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Natural Oscillations of the Bay of Fundy[1]

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ABSTRACT

The one-dimensional channel equations are integrated numerically to determine the periods and structures of the lowest three longitudinal modes of free oscillations of the Bay of Fundy. It is found that the period of the lowest mode is 9.047 hr rather than somewhere in the vicinity of the M_2 tidal period of 12.42 hr, as has been supposed hitherto. This estimate of the free period is obtained without considering the effects of rotation and friction, but an approximate treatment of these effects shows that their effect on the period is very small — rotation tends to *decrease* the period by less than 3% and friction tends to *increase* it by 1% at the most. The periods of the second and third modes are found to be 5.383 and 3.475 hr. The modal structures and their modification produced by rotational effects are presented.

INTRODUCTION

One of the most conspicuous features of the tidal oscillations in the Bay of Fundy is their very large amplitude at the head of the Bay. The range of the spring tides is known to reach values as high as 50 ft at the head of the Bay and for this reason they have sometimes been referred to as "the greatest tides in the world" (Marmer, 1926). The popular supposition is that the Bay acts as a quarter-wave reasonator with a natural period close to that of the semi-diurnal component (M_2) of the lunar tide which accounts for most of the tidal range observed in the Bay of Fundy. Rough estimates based on Merian's formula for the period in a bay with Rayleigh's correction for the open end have placed the natural period of the lowest mode between 11.6 and 13 hr by Honda et al. (1908) and at 11.06 hr by Proudman (1952). Since the period of the M_2 component is 12.42 hr, it is naturally assumed that the proximity of the natural period to the forcing period is generating the large amplitudes at the head of the Bay by resonance. Rather surprisingly, however, no one seems to have made an attempt to determine the natural period of the Bay taking into account, more precisely, the depth and width variations in the Bay as remarked by Sverdrup et al. (1942).

The purpose of the present investigation is to determine by a more accurate method the natural periods of some of the lowest longitudinal modes of the Bay of Fundy. The periods of the lowest three longitudinal modes of the Bay are obtained by a direct numerical integration of the well-known channel equations. The effects of bottom friction and earth's rotation are ignored in this study as a first approximation. Since the Bay of Fundy is a

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relatively narrow bay, the effect of rotation on the periods of oscillation would be very small, the primary affect of rotation being on the structure of the mode. This effect can be approximately treated on a Kelvin wave-type hypothesis, which transforms an otherwise standing wave into an amphidromic system. An approximate treatment of the effect of bottom friction shows that it affects the period only slightly.

In the next section, the channel equations and the method of solution are given. The results of the computations are then presented, followed by some approximate considerations of the effects of rotation, friction, and mean level variations of the Bay, and finally conclusions. In addition to these various considerations of the natural or free-oscillations, a calculation is made to determine the co-oscillating response of the Bay for M_2 period (12.42 hr) imposed at the mouth of the Bay with unit amplitude for the height field. The calculated amplification of the M_2-tide from the mouth to the upper reaches of the Bay is compared with observed amplification and the agreement between them is found to be good, thus verifying the accuracy of the numerical model used. These results are given in the appendix. Even though the treatment of the problem in this paper follows closely that adopted by Platzman and Rao (1964) in their study of the free oscillations of Lake Erie, most of the mathematical details are retained here for the sake of completeness.

THE CHANNEL EQUATIONS AND THE INTEGRATION PROCEDURE

The linearised momentum and mass equations for flow in a channel of gradually varying section are

$$\frac{\partial \hat{Q}}{\partial t} = -gS\frac{\partial \hat{\zeta}}{\partial x} \qquad \ldots\ldots(2.1)$$

$$\frac{\partial \hat{\zeta}}{\partial t} = -\frac{1}{b}\frac{\partial \hat{Q}}{\partial x} \qquad \ldots\ldots(2.2)$$

where $\hat{Q}(x,t)$ is the volume transport through a vertical section of the channel; $S(x)$ is the area of the section; $\hat{\zeta}(x,t)$ is the mean water level across the section relative to the undisturbed level; $b(x)$ is the surface breadth of the section; and g is the acceleration due to gravity. The x-axis is locally tangent to the principal axis of the channel, which may be considered as coinciding with the direction of flow near the channel center.

Since we are looking for the free-oscillations of the system, let

$$\hat{Q}(x,t) = Q(x)\sin\sigma t;\quad \hat{\zeta}(x,t) = \zeta(x)\cos\sigma t \qquad \ldots\ldots(2.3)$$

where σ is the oscillation frequency (period is $2\pi/\sigma$) and $Q(x)$, $\zeta(x)$ are the space dependent normal mode functions of the basin. Substitution of equation 2.3 into equation 2.1, 2 results in:

$$\sigma Q = -gS\frac{d\zeta}{dx} \qquad \ldots\ldots(2.4)$$

$$\sigma\zeta = \frac{1}{b}\frac{dQ}{dx} \qquad \ldots\ldots(2.5).$$

The boundary conditions of the problem are

$$Q = 0 \text{ at the head of the Bay,} \qquad \ldots\ldots(2.6)$$

$$\zeta = 0, Q \text{ arbitrary at } x = 0, \text{ the mouth of the Bay} \qquad \ldots\ldots(2.7).$$

Equations 2.4, 5, 6, 7 constitute a characteristic value problem for the frequencies σ.

The Bay of Fundy (Fig. 1) consists of a more or less rectangular-shaped body at the mouth. As one proceeds into the Bay, it splits into Minas Basin

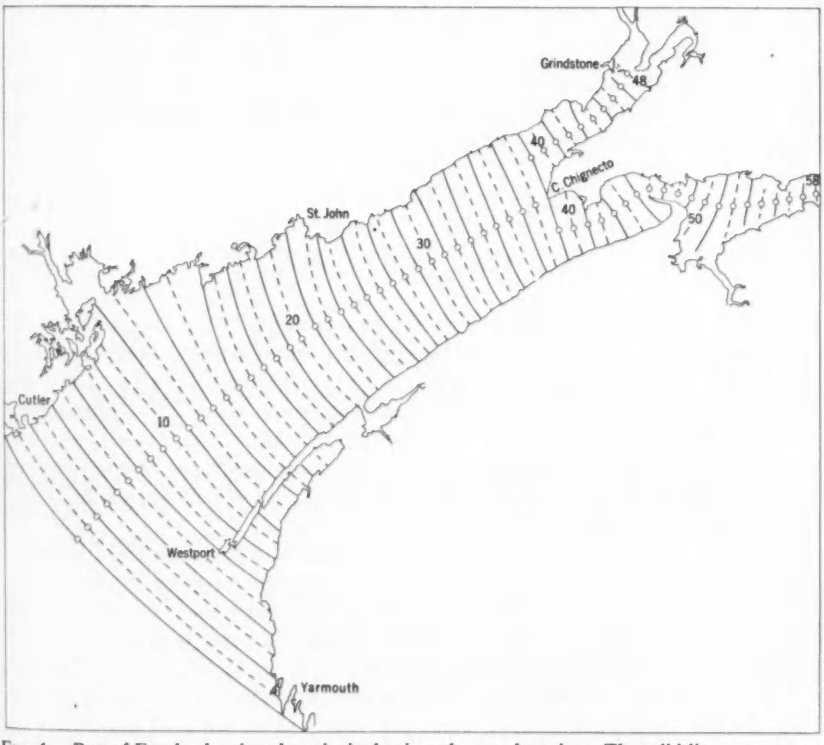

FIG. 1. Bay of Fundy showing the principal axis and normal sections. The solid lines represent ζ sections and the broken lines Q sections.

(lower branch in Fig. 1) and Chignecto Bay (upper branch). In this calculation, the mouth of the Bay of Fundy was taken as the line joining Machias on the Maine side to Chebogue Point on the Nova Scotia side. South of this point, the Bay fans out sharply and this seems to establish the line connecting Machias and Chebogue Point as a southern limit to what may be considered as the Bay of Fundy system. In the work of Honda et al. (1908) quoted earlier, the period of 11.6 hr was obtained by considering the mouth of the Bay to be between Machias and Yarmouth which is very close to what is considered here.

The dotted line in Fig. 1 shows the principal axis of the Bay of Fundy. In the calculations to be described below, ζ values are calculated at each of 29 sections identified in Fig. 1 by solid lines and values of transport Q are calculated at each of 29 sections identified by broken lines. The sections are drawn to intersect the principal axis at uniformly spaced points, the distance between two successive ζ or Q sections being $\frac{1}{3} \times 10^5$ ft (6.3 statute miles). The section area S was determined at each Q section and the surface width b at each ζ section using the Canadian Hydrographic Survey charts 4010 (scale 1:200,000) and 4011 (scale 1:300,000). The results $b(x)$ and $S(x)$ are shown in Fig. 2.

There are several ways of solving the system of equations 2.4, 5 (see Defant, 1961). The simplest and the most straight-forward is the direct numerical integration of these equations in which the continuous equations 2.4, 5 are replaced by central difference equations:

$$\zeta_{i+1} = \zeta_{i-1} - \sigma C_i Q_i \quad i = 2(2)56 \qquad \ldots\ldots(2.8)$$

$$Q_{i+1} = Q_{i-1} - \sigma d_i \zeta_i \quad i = 3(2)57 \qquad \ldots\ldots(2.9)$$

where $C_i = \Delta x/gS_i$, $d_i = -b_i \Delta x$. To solve equation 2.8, 9 we assign $Q_2 = 10^8$ ft^3 per second (an arbitrary value because the equations are homogeneous); then a trial value is given to σ, and ζ_3 is computed from equation 2.8 using the condition $\zeta_1 = 0$. Then Q_4 is computed from equation 2.9. By alternate use of equations 2.8, 9, we arrive at Q_{58} which should be zero, since this is the boundary condition of the problem. If $Q_{58} \neq 0$, the guess value of σ is changed and the procedure is repeated until, by interpolation or otherwise, a sufficiently accurate value of σ is obtained.

There is a departure from the standard procedure just described in order to take into account Chignecto Bay. At section 39 which passes through Cape Chignecto, Minas Channel and Chignecto Bay branch out from the Bay of Fundy. Hence, the Bay of Fundy system considered here, is split into three distinct regions: the main channel for which $1 \leq i \leq 39$, Minas Channel and Minas Basin for which $39 \leq i \leq 58$, and Chignecto Bay for which $39 \leq i' \leq 48$. The channel equations, in principle, are then formulated separately in each region with joining conditions for the continuity of ζ and the transport

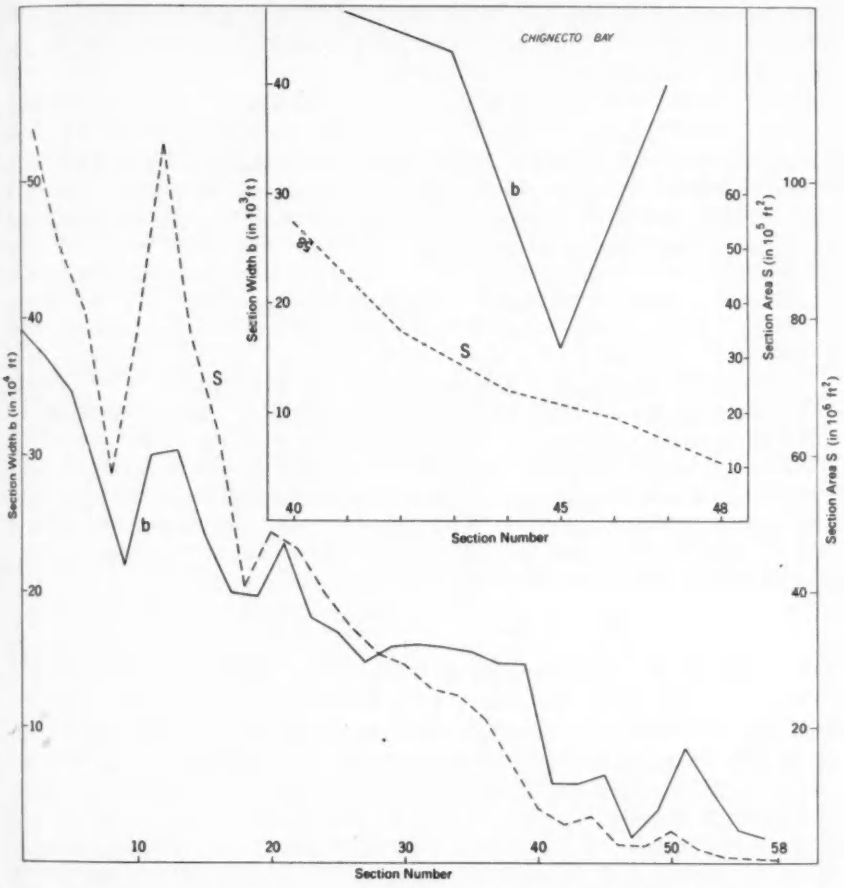

FIG. 2. Variation of width (b) and cross-sectional area (S) in the Bay of Fundy. Inset represents the data for Chignecto Bay.

at section 39, which is common to all three regions. Using primes to denote quantities in the Chignecto Bay, what we actually have instead of equation 2.8, 9 is the following:

$$\zeta_{i+1} = \zeta_{i-1} - \sigma\, C_i\, Q_i \begin{pmatrix} i = 2(2)38 \\ 40(2)56 \end{pmatrix} \qquad \ldots\ldots(2.10)$$

$$Q_{i+1} = Q_{i-1} - \sigma\, d_i\, \zeta_i \begin{pmatrix} i = 3(2)37 \\ 41(2)57 \end{pmatrix} \qquad \ldots\ldots(2.11).$$

At section 39 we get

$$\sigma\, b_{39}\zeta_{39}\Delta x = Q_{40} + Q'_{40} - Q_{38} \qquad \ldots\ldots(2.12)$$

In Chignecto Bay:

$$\zeta'_{i+1} = \zeta'_{i-1} - \sigma\, C'_i Q'_i,\ i = 40(2)46 \qquad \ldots\ldots(2.13)$$

$$Q'_{i+1} = Q'_{i-1} - \sigma\, d'_i,\ \zeta'_i,\ i = 41(2)47 \qquad \ldots\ldots(2.14).$$

Equations 2.10, 11 and 2.13, 14 are similar to equations 2.8, 9. However, neither equation 2.11 nor 2.14 is applied at section 39. There, equation 2.12 expresses the continuity of transport between the three regions. An additional condition joining the three regions is

$$\zeta_{39}(\text{Main}) = \zeta_{39}(\text{Minas}) = \zeta'_{39} \qquad \ldots\ldots(2.15)$$

for continuity of height.

The procedure now is as follows. Starting with $\zeta_1 = 0$, $Q_2 = 10^8$ and an estimate for σ, we integrate equation 2.10, 11 until we reach section 39. In order to cross this section, we need to know Q_{40} from equation 2.12, which involves Q'_{40} another unknown. Q'_{40} can be obtained by solving equation 2.13, 14 for Chignecto Bay. It may be noticed that the oscillation in Chignecto Bay may be considered as a (forced) co-oscillation with the imposed frequency σ and an imposed elevation ζ'_{39} at the mouth. Then noting that $Q'_{48} = 0$ we can solve for ζ' and Q' in terms of ζ_{39} $(=\zeta'_{39})$:

$$\begin{array}{cc} \zeta'_i = A_i\, Q'_{i-1} & Q'_i = B_i\, \zeta'_{i-1} \\ A_i \equiv (\sigma d'_i + B_{i+1})^{-1}\ ; & B_i \equiv (\sigma C'_i + A_{i+1})^{-1} \\ i = 47(2)41 & i = 46(2)40 \end{array} \qquad \ldots\ldots(2.16)$$

with $B_{48} = 0$. The last equation of the above system then expresses Q'_{40} in terms ζ'_{39}. Then, using equation 2.12, Q_{40} is obtained. Once again we use equation 2.10, 11 to march up to the head of the Bay and complete one cycle of integration from the mouth to the head of the Bay. Convergence to $Q_{58} = 0$ is obtained by adjusting the value of σ appropriately. In this way, one obtains the value of σ and the associated transport and profile distribution in the Bay.[2]

NUMERICAL RESULTS

The period values are calculated for the lowest three longitudinal modes. These values are given below:

Mode (n)	Period (*hours*) T_n	T_1/T_n
1	9.047	1
2	5.383	0.595
3	3.475	0.384

[2]A similar analysis was made for Mary's Bay close to the mouth of the Bay of Fundy and its effect was found to be negligible. Hence, in the final calculations, the presence of Mary's Bay was ignored.

Notice that the ratio of the periods of the second and third modes to that of the first mode are 0.595 and 0.346 instead of $\frac{1}{3}$ and $\frac{1}{5}$, which is what one obtains in the case of a basin of uniform depth by the use of the appropriate Merian's formula ($T_n = 4L/[(2l+1)\sqrt{gD}]$; $l \equiv n-1$). The various approximate values for the period of the lowest mode obtained by the use of Merian's formula are given already in the introduction. If, instead of treating the entire Bay of Fundy as a rectangular bay of uniform depth, we represent it as a connected system consisting of three sections (main channel, Chignecto Bay, and Minas Channel) each having a constant depth and breadth (an appropriate average value in each part), then the values of the lowest three modes are found to be 10.045, 6.439, and 2.731 hr. The average values of breadth (b) and cross-sectional area (s) used in these calculations are given in Table I. If

TABLE I. Average dimensions of the various basins.

	Length (L) (ft)	Breadth ($\bar{b}$) (ft)	Cross-section ($\bar{S}$) (ft^2)	$\bar{D}=\bar{S}/\bar{b}$ (ft)
Main	6.5×10^5	2.263×10^5	5.39×10^7	238.2
Chignecto	1.5×10^5	4.062×10^4	2.9×10^6	71.4
Minas	3.166×10^5	4.648×10^4	3.4×10^6	73.1

any idealisation of the Bay is intended to be done, this certainly is a better way of doing it rather than representing it as a single basin of uniform depth and breadth. In any case, the period of the lowest mode of the Bay of Fundy is not close to the period of the M_2 component for significant resonance effect to come into play. As a matter of fact, Redfield (1950) and Ippen and Harliman (1958) concluded from an analysis of tidal data that the Bay of Fundy is *not* in such a highly resonant state. The tidal amplification obtained in the Bay is also realisable in other basins like, for example, Long Island Sound. The reason for the large range of the tide in the upper reaches of the Bay of Fundy is due to the high range of tide at the entrance of the Bay itself. The estimate of 9.047 hr for the period of the lowest mode is only slightly affected by considerations of friction and rotation as will be briefly shown later on.

Figure 3 shows the structure of the free modes in terms of the perturbation height (ζ). The solid line in these pictures shows the height distribution from the mouth of the Bay into Minas Channel and Minas Basin. The dotted line starting from section 39 and going up to section 47 gives the height distribution in Chignecto Bay. At the mouth of the Bay all these modes have a node (section 1). The lowest mode is a monotonic increasing function of the distance from the mouth of the Bay with no nodal lines in the interior. The second mode has one nodal line between sections 43–44 (in the Minas Channel area) and the third mode has two nodal lines (one at section 34 and the other at section 49) in the interior of the basin as shown in this figure. Notice the

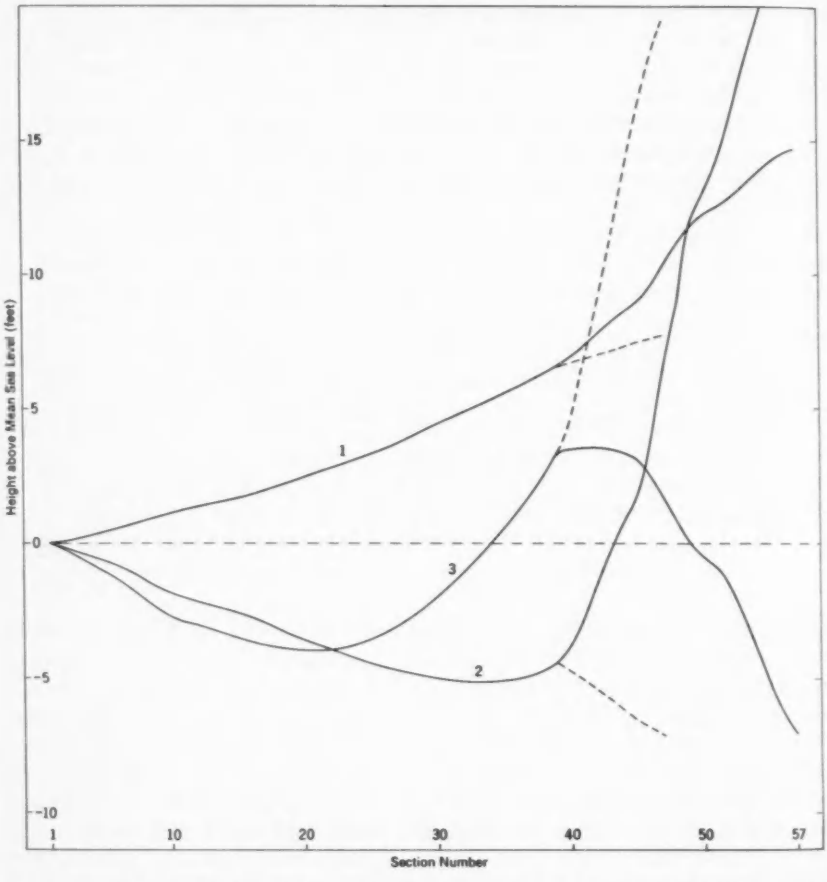

FIG. 3. Structure of the lowest longitudinal modes in terms of perturbation height (ζ). The broken short line between sections 39 and 57 represents the structure in the Chignecto Bay.

high magnification in Chignecto Bay for mode 3. This results from the fact that the lowest mode in Chignecto Bay appears to have a free period of 3.478 hr according to Merian's formula ($4L/\sqrt{gD}$) using the data of Table I. Since the period of the third mode is 3.475 hr, a condition of resonance is being created in the co-oscillation of Chignecto Bay.

Figure 4 shows the volume transport for the three modes considered here. This figure shows that the maximum transport occurs at the mouth.

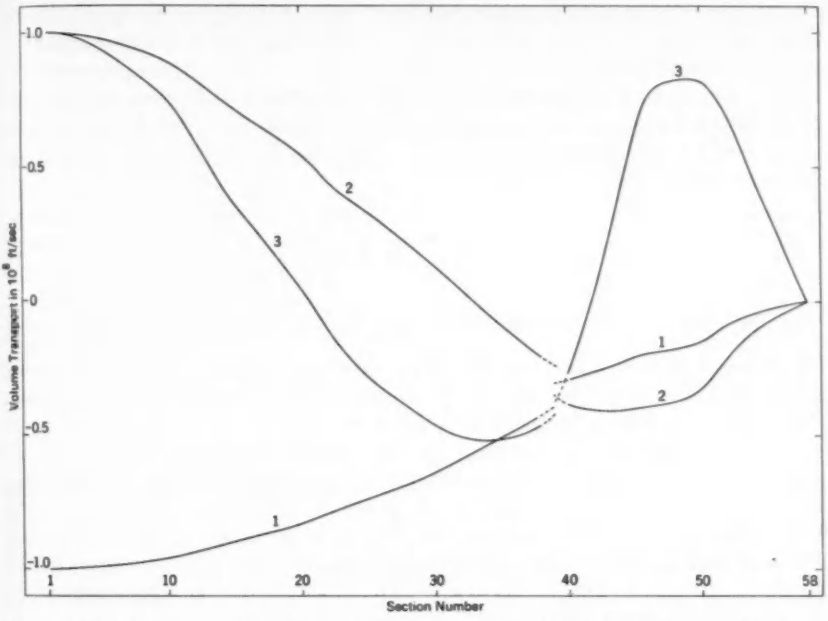

FIG. 4. Modal structures in terms of the volume transport (Q). The jump at section 39 represents the transport into Chignecto Bay.

APPROXIMATE CONSIDERATION OF ROTATION, FRICTION, AND MEAN LEVEL VARIATION

ROTATION

An exact treatment of the effects of rotation in a rectangular bay is a complicated problem, requiring the formulation in two space dimensions. The calculated nonrotating period of the bay (9.047 hr) is removed far enough from the inertia period 16.92 hr, to justify ignoring rotation as a first approximation. In addition, the effect of rotation *on the period* is further restricted in view of the narrowness of the Bay of Fundy over the main part of which the length to breadth ratio of the order of 1 to $\frac{1}{3}$. The only study that seems to have been made on the effect of rotation on the free periods of a rectangular bay is that of Van Dantzig and Lauwerier (1960). For the case of a bay whose length L is twice its breadth, the approximate value for the frequency σ of the lowest mode is given by:

$$\sigma = \sigma_0 + 0.504 \frac{f^2 L}{2\pi C} + 0(f^4)$$

where σ_0 is the lowest nonrotating frequency given by $\sigma_0 = \pi C/2L$. C here is $\sqrt{gD}$, the speed of long gravity waves and f is the coriolis parameter. The

above equation is rather curious because it shows that the frequency of the lowest mode in the case with rotation is increased over its corresponding value in the nonrotating case — hence, the period of the lowest nonrotating mode is reduced by rotation. This result is opposite of what normally happens in completely enclosed basins. In any case, if we take $\sigma_0 = 2\pi/(9.047 \times 3600)$ where 9.047 is the period we calculated in hours, then L/C will be 8143.3 sec. We then obtain with $f = 10^{-4}$

$$\frac{\sigma L}{C} = \frac{\pi}{2} + 0.053.$$

This then shows that the nondimensional period $T(= 2\pi/\frac{\sigma L}{C})$ in the rotating case is 3.87, whereas it was 4 in the case of no rotation. Hence, the period change is 3.25% for a bay of 1 to ½ length to breadth ratio. In the case of a 1 to ⅓ bay, the change in period would be even less.

Even if the effect of rotation on the period of oscillation is small, its effect on the structure of the oscillation is important because the standing nature of the oscillation is lost in the presence of rotation, however small. This effect may be calculated by the following standard approximation that was used extensively in other investigations (see for example Mortimer (1955), Platzman and Rao (1964)). We assume that the transverse slope is given by the geostrophic equation

$$f\,\hat{Q} = -\,gS\,\frac{\partial \bar{\zeta}}{\partial y}$$

where $\bar{\zeta}$ is the added surface displacement and $\hat{Q}$ is the longitudinal mass-transport that is already calculated. Integration of this equation across the channel, coupled with the assumption that the geostrophic effect is distributed equally between the north and south shores yields the following expressions for the height along the north and south shores:

$$\hat{\zeta}_S = A(x)\cos\sigma(t-t_s),\ \hat{\zeta}_N = A(x)\cos\sigma(t-t_n)$$

where

$$A(x) \equiv (\zeta^2 + \frac{f^2b^2}{4g^2S^2}\,Q^2)^{1/2}$$

$$t_s(x) = \sigma^{-1}\tan^{-1}(\frac{fbQ}{2gS\zeta});\ t_n = T - t_s$$

where T is the period $2\pi/\sigma$ and t_s, t_n are the phases of high water along the south and north shores.

The result of the foregoing treatment is to transform the standing mode into an amphidromic system with positive phase propagation — that is, the

cotidal lines rotate in a counter-clockwise direction about an amphidromic point located in the interior of the Bay. This, then, means that it is no longer possible to have a nodal line fixed in space for all time as we had in the case of $f = 0$. Figure 5 shows the amplitude distribution on the shore for the lowest two modes. Notice that at the nodal points for these modes when there is no rotation (see Fig. 3), we now have a nonzero amplitude.

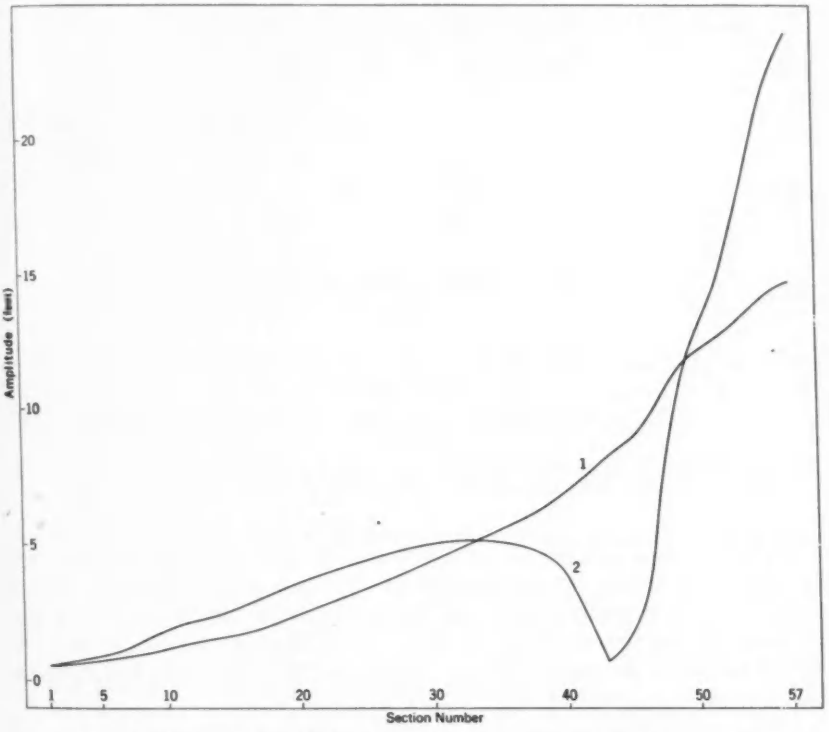

FIG. 5. Computed amplitudes of the first and second modes with rotation.

Figure 6 shows the affect of rotation on the phase propagation of the lowest two modes along with the corresponding situation for the no-rotation case. For the lowest mode, if the time of high water in the case of no rotation is designated by $t = T/2$ (T being the period of the mode), then with rotation the high water starts at the mouth of the bay on the south shore at $t = T/4$, propagates in a counter-clockwise sense reaching the head of the bay (section 58) at $t = T/2$. Then the high-water phase continues to travel on the north shore and returns to the mouth of the bay at $t = 3T/4$. That is, for the lowest mode it takes half a period for the phase of high water to start at the mouth

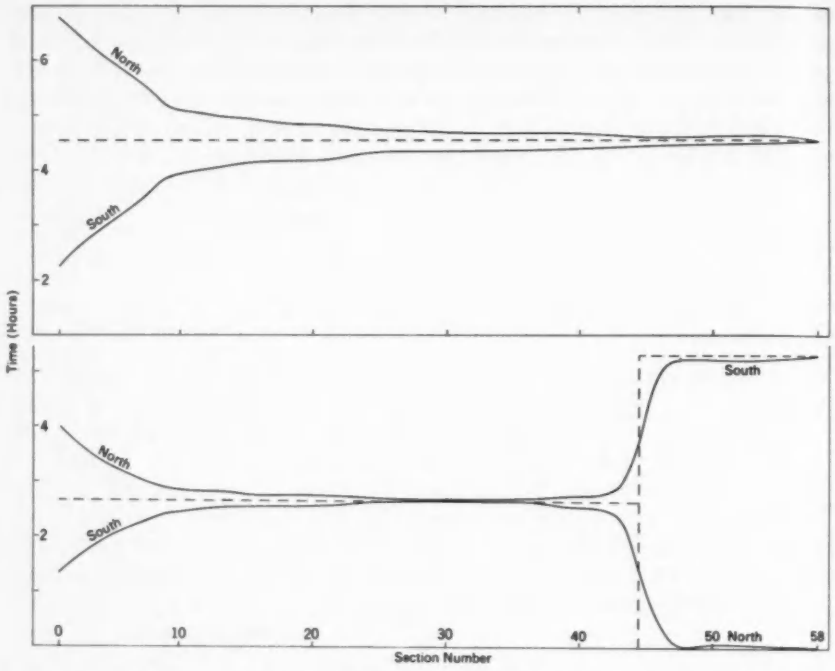

FIG. 6. Computed phase of the first mode (*upper curve*) and the second mode (*lower curve*) with rotation (*solid line*) and without rotation (*broken line*) in the Bay of Fundy.

on the south shore and return to the mouth on the north shore of the bay. For the second mode, the propagation of the phase of high water is similar to that of the first mode between the mouth of the Bay and section 33, where this mode has an anti-node for the height field. Between section 33 and the head of the Bay, the propagation of phase goes through a complete counter-clockwise cycle in one period.

FRICTION

In the calculations described so far, the effect of bottom friction has been ignored as a justifiable first approximation. We shall consider here the effect of friction in an approximate manner aimed primarily at obtaining its effect on the period increase. The integrated momentum and mass equations with the bottom friction included are:

$$\frac{\partial \hat{Q}}{\partial t} = -gS\frac{\partial \hat{\zeta}}{\partial x} - \frac{Fb}{\rho}; \quad \frac{\partial \hat{\zeta}}{\partial t} = -\frac{1}{b}\frac{\partial \hat{Q}}{\partial x} \qquad \ldots\ldots(4.1, 2)$$

where F is the mean bottom stress across a section. The most reasonable

assumption about F is the quadratic stress law $F_2 = k\rho|\hat{U}|\hat{U}$ where k is the coefficient of skin friction. However, this representation of F introduces complications due to nonlinearities. To avoid this, one normally resorts to the linear stress law $F_1 = k\rho U\hat{U}$ where U is a constant velocity. Then (4.1) gives

$$\frac{\partial \hat{Q}}{\partial t} = - gS \frac{\partial \hat{\zeta}}{\partial x} - \frac{kUb}{S} \hat{Q} \qquad \ldots\ldots(4.3).$$

Now let

$$\hat{Q} = \mathrm{Im}(-Q(x)e^{i\sigma t}), \hat{\zeta} = \mathrm{Re}(z(x)e^{i\sigma t}) \qquad \ldots\ldots(4.4).$$

Substitution of 4.4 in 4.2, 3 results in:

$$\partial\zeta = \frac{1}{b}\frac{dQ}{dx} \qquad \ldots\ldots(4.5a)$$

$$\sigma Q = - gS\frac{d\zeta}{dx} + ikU\frac{b}{S}Q \qquad \ldots\ldots(4.5b).$$

In the case of a rectangular bay of length L and uniform depth D, the above equations result in an equation for damped simple harmonic oscillations with $\sigma = i\lambda \pm \nu$, the decay rate λ and the frequency ν being given by

$$\lambda = kU/2D \; ; \; \nu = \sqrt{\nu_0^2 - \lambda^2}$$

where $\nu_0 = \pi\sqrt{gD}/2L$ is the frequency of the inviscid oscillations. Hence the period ratio is given by

$$\gamma = \frac{\nu_0}{\nu} = \sqrt{1 + (\beta/2\pi)^2}$$

where $\beta \equiv 2\pi\lambda/\nu$ is the decay modulus.

For a general channel, equation 4.5 may be integrated numerically if a value is assigned to kU. On the other hand, one may try to consider the effect of friction approximately by treating it as a perturbation quantity in equation 4.5. It is the latter procedure that has been followed here. Briefly, it consists in expanding ζ, Q, and σ in powers of ikU:

$$\begin{aligned} \zeta &= \zeta_0 + (ikU)\,\zeta_1 + \\ Q &= Q_0 + (ikU)\,Q_1 + \\ \sigma &= \sigma_0 + (ikU)\,\sigma_1 + (ikU)^2\,\sigma_2 + \end{aligned} \qquad \ldots\ldots(4.6).$$

Substitution of equation of 4.6 into 4.5 leads to pairs of equations for successive determination of ζ_0, Q_0, ζ_1, Q_1 etc. together with σ_0, σ_1, ... The results of

this analysis may be conveniently represented in terms of perturbations of the integrals:

$$K = \tfrac{1}{2}\int_0^L \frac{Q^2}{S}\,dx\ ,\ W = \tfrac{1}{2}\int_0^L \frac{Q^2}{S^2}\,bdx$$

which, respectively, are proportional to the kinetic energy and the dissipation rate in the basin. The first result of the perturbation is:

$$\sigma_1 = \tfrac{1}{2}\frac{W_0}{K_0} \qquad \ldots\ldots(4.7)$$

which is conveniently obtained in terms of Q_0 that is already obtained from the inviscid analysis. So to the first order in ikU (see equation 4.6) the friction does not modify the period, but only gives the damping rate $kU\sigma_1$. The result of the perturbation analysis, to the second order gives:

$$\sigma_2 = \tfrac{1}{2}\frac{\sigma_1^2}{\sigma_0} + \tfrac{1}{4}\frac{W_1K_0 - W_0K_1}{K_0^2} \qquad \ldots\ldots(4.8)$$

where

$$K_1 = \int_0^L \frac{Q_0Q_1}{S}\,dx\ ,\ W_1 = \int_0^L \frac{Q_0Q_1}{S^2}\,bdx.$$

The above integrals involve Q_1 which can be obtained by solving the first order perturbation equations. To the second order, then, the frequency is given by

$$\sigma = \sigma_0 - (kU)^2\sigma_2$$

so that the period has been increased over the inviscid period. The period ratio σ_0/σ is then

$$\gamma = 1 + k^2U^2\frac{\sigma_2}{\sigma_0} \qquad \ldots\ldots(4.9)$$

The numerical values of W_0, K_0, W_1, and K_1 for the lowest mode in the Bay of Fundy are:

$$\begin{aligned} W_0 &= 0.3465 \times 10^{12}\ \text{ft}^4\ \text{sec}^{-2} \\ W_1 &= 0.1538 \times 10^{14}\ \text{ft}^3\ \text{sec}^{-1} \\ K_0 &= 0.5018 \times 10^{14}\ \text{ft}^5\ \text{sec}^{-2} \\ K_1 &= -0.1389 \times 10^{14}\ \text{ft}^4\ \text{sec}^{-1} \end{aligned} \qquad \ldots\ldots(4.10).$$

Using these values in equation 4.7 we obtain $\sigma_1 = 0.0034\ \text{ft}^{-1}$, $\sigma_2 = 0.108\ \text{sec/ft}^{-2}$. If we take the standard value of $k = 0.0025$ for the skin friction coefficient and maximum value for U of 1.5 ft/sec^{-1}, the decay modulus

$2\pi k U\ \sigma_1/\sigma_0$ is 0.416, a small value. The increase of period as given by equation 4.9 is less than 1% with the above values of σ_2, k, U. This justifies indirectly the ignoring of friction in our calculations, at least as a first approximation.

Effect of Mean Depth Variation

The bathymetric data used in establishing the areas of vertical sections are taken from the Canadian Hydrographic Charts 4010 and 4011, which are based on a low-water reference. From the data obtained in equation 4.10, it is possible to estimate the change in the calculated periods due to any changes in the mean level of the Bay. If δD represents a small variation of the mean level, the corresponding variation in the sectional area is $\delta S_i = b_i\, \delta D$. This causes a small change in the sectional area S of equation 2.4. The preceding analysis — now done without the friction term — enables us to estimate the change in period due to the change in S. To do this we expand for σ,

$$\sigma = \sigma_0 + (\delta D)\sigma_1 +$$

and similarly for Q and ζ. The first order effect then is found to be

$$\sigma_1 = \sigma_0 \frac{W_0}{2K_0}.$$

The period ratio $\gamma = \sigma_0/\sigma$ then is

$$\gamma = 1 - \tfrac{1}{2} \frac{W_0}{K_0}\, \delta D + 0(\delta D)^2.$$

From the calculated values, we have $W_0/2K_0 = 0.00345$ ft^{-1} for the lowest mode. Hence the percentage change in the period is $-0.345\delta D\%$ if δD is given in feet.

CONCLUSIONS

The period of oscillation for the lowest mode in the Bay of Fundy is found to be 9.047 hr, which is far removed from the values that have been assigned to this period in the past on the basis of Merian's formula. An approximate treatment for the influence of rotation and friction showed that they will not significantly effect the period estimate of 9.047 hr.

The calculated period of 9.047 hr for the lowest mode is removed far enough from the period of M_2 (12.42 hr) for any significant resonance effects to come into play. This conclusion is in agreement with that of Redfield (1950) and Ippen and Harliman (1958). The amplification of M_2 tide at head of Chignecto Bay is found to be about 2.5 as shown in the appendix and this value is in agreement with the observed value. This calculation also bears out the accuracy of the numerical model used.

ACKNOWLEDGMENTS

I am grateful to Mr David Greenberg for doing the planimetering work to obtain the breadth and sectional areas and for doing the necessary programming for the computer. I am also indebted to Mr Bryce Ramlo for help with some additional programming and calculations and Mr Maurice Isabelle for drafting the diagrams.

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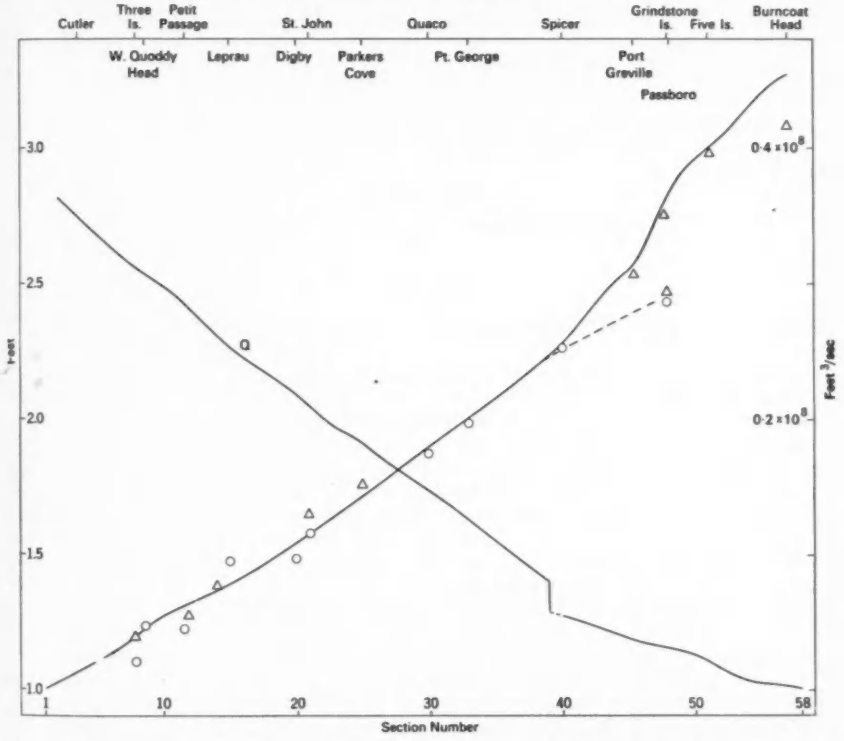
(APPENDIX *follows*)

APPENDIX

Co-oscillating Tides in the Bay of Fundy

As a check on the numerical model used for the calculation of the free periods, the co-oscillating response of the Bay is calculated for the M_2 tidal period (12.42 hr) imposed with unit amplitude for ζ at the mouth. The integration procedure is the same as the one described in section 2 except that we now prescribe $\sigma = 2\pi/$ (12.42 hr) and start the integration with $\zeta(1) = 1$ and a guess value for Q(2). Then the integration procedure is iterated through a sequence of Q(2) values aimed at convergence to a zero value of Q(58).[3] The result of this calculation is shown in App. Fig. 1 which shows the transport Q and profile ζ as a function of the distance down the Bay. In the same diagram we have shown by the open circle the observed values of tidal amplification taken from Table I of Ippen and Harliman (1958) — the amplification factors given by these authors are with respect to a unit amplitude at Cutler, Me. Since the amplitude at Cutler in our calculations is 1.07, we have multiplied all values of the amplification factors given by Ippen and Harliman by 1.07 and plotted them on App. Fig. 1. The points marked as an open



App. Fig. 1. Computed M_2 co-oscillation in the Bay of Fundy. ○ indicate observed values taken from Ippen and Harliman; △ are observed values taken from Yuen (1967).

[3]In calculating the co-oscillation tide, it is more convenient to proceed from the head of the Bay to the mouth starting the integration with the conditions Q(58) = 0, $\zeta(57) = 1$. Then only one pass through the channel is sufficient to solve the problem. However, since the integration procedure is already set up for the free oscillation problem to procede from the mouth to the head of the Bay, we retained the same path for the co-oscillation problem, even though it meant going through a few iterations before Q(58) can be reduced to zero.

triangle represent data taken from Table I.1 of Yuen (MS, 1967). His data was normalised with the amplitude at Westport and then multiplied by 1.2 since the amplitude of the M_2-co-oscillation along the section passing through Westport in our calculation is 1.2. It is seen that the general agreement between computed and observed values is very good except for a few stations near the mouth of the Bay. The discrepancies are probably due to our ignoring the effects of the various Bays and passages in the vicinity of the stations and the drastic smoothing of the shore line. The data point corresponding to Burncoat Head (near section 57) is not in good agreement with the calculated value. This can be due to the fact that we artificially closed off the Minas Basin at section 58, where in fact a discharge still takes place through section 58. Moreover, one has to bear in mind that the values obtained from the channel equations are more of the nature of average values along a given section and these are being compared here with exact values at given stations. In spite of this fact, the agreement between observed and calculated amplitudes is good and suggests that the important characteristics of the Bay have been faithfully incorporated in our numerical model.

Sounding Response of the Kokanee and Sockeye Salmon[1]

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ABSTRACT

When frightened, sockeye and kokanee salmon dive into deeper waters. This is termed the "sounding response."

The sounding response in these fish is accompanied by the expulsion of gas from the swimbladder via the pneumatic duct. This gas loss is active and results from the contraction of the circular muscle fibers in the wall of the swimbladder.

Gas loss on sounding is reduced or obviated after exposure to adrenergic blocking agents and enhanced in fish treated with sympathomimetics. Circular muscle fibers in intact and isolated strips of swimbladder contracted in response to sympathomimetics, but this response was prevented after adrenergic blockade. These results suggest the concentration of the swimbladder and associated gas expulsion is under adrenergic control.

In untreated fish, an average pressure of 28.1 mm Hg was required to force gas out of the swimbladder through the pneumatic duct. In antropine-treated fish this duct-release pressure fell to an average of 0.2 mm Hg. This suggests constriction of the pneumatic duct is under cholinergic control.

Histologically, the pneumatic duct was continuous with the oesophagus and extended as a convoluted duct to the anterior end of the swimbladder. Smooth muscle fibers surrounded the duct along its length and some striated fibers were present at the point where the duct joined the oesophagus. Occlusion of the pneumatic duct may be through the contraction of either of these types of fibers.

The term "Gasspuckreflex" as originally used by Franz described the loss of gas from the swimbladder of physostomes in response to decompression. The authors suggest this term be reserved for this passive loss of gas, and not applied to fright-induced gas loss, resulting from active contraction of the bladder wall.

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INTRODUCTION

WHEN STARTLED, fish behave in different ways. Even closely related species display markedly different patterns of escape behaviour. When alarmed, schools of pink salmon fry (*Oncorhynchus gorbuscha*) scatter wildly in different directions, but remain near the surface, whereas sockeye (*O. nerka*), coho (*O. kisutch*), and chum (*O. keta*) fry dart toward the bottom (Hoar, 1958). Migrant sockeye and young kokanee (also *O. nerka* but lacustrine throughout life) characteristically expel gas from their swimbladders when they sound or dive into deeper waters. In this sounding behaviour the fright response of kokanee and sockeye differs from that of other species of *Oncorhynchus*. In the sockeye, sounding with loss of gas continues throughout its life. Fishermen watch for this during commercial fishing operations. When trapped in purse seines, salmon are alarmed sufficiently to sound and the sockeye catch may be forecast by the extent of bubbles rising to the ocean surface.

The sounding response of young kokanee and migrant sockeye (smolts) can be observed readily under simple experimental conditions. When startled, the fish sound quickly to the bottom of an aquarium leaving an obvious trail of bubbles. Kokanee and sockeye may be observed to gulp air on return to the surface after sounding. The aim of the present study was to investigate this sounding response. The amount of gas released was calculated from the change in density of the fish on sounding. The volume of the swimbladder and release pressure of the pneumatic duct were determined. The swimbladder and pneumatic duct were examined histologically, and an attempt was made to elucidate the nature of the nervous control of the duct and bladder through the use of pharmacological agents.

MATERIALS AND METHODS

The study was conducted on young kokanee of Kootenay Lake origin and on sockeye smolts trapped during their seaward migration from Cultus Lake, British Columbia. The kokanee were reared from eggs and tested as yearlings weighing from 9 to 16 g. With rare exceptions, the sockeye smolts were 1 year old and ranged in size from 3 to 12 g, with the majority 4–6 g. All studies were conducted at 10 C.

SOUNDING RESPONSE

The buoyancy of sockeye and kokanee in the holding containers was determined by stunning individual fish electrically and measuring their density. Such fish were invariably at neutral buoyancy. For this reason the increase in density of the test fish is attributed to gas loss on sounding as a consequence of being captured and placed in an illuminated cylinder.

The extent of gas loss on sounding was determined as follows. Individual fish were netted from the holding containers and placed in the 4-liter, cylindrical glass jar shown in Fig. 1. A kokanee or sockeye treated in this way always sounds to the bottom and then circles the bottom, all the while

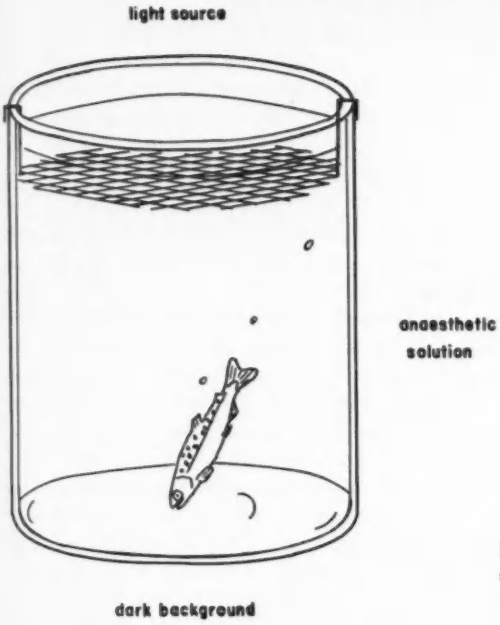

FIG. 1. Apparatus used to induce the sounding response in kokanee and sockeye salmon.

releasing small bubbles of gas. Gas loss is usually completed within 30 sec. In preliminary tests fish were stunned electrically 2 min after sounding and then removed for density determination. Subsequently it was found to be simpler and more satisfactory to use an anaesthetic solution of tricaine methanesulfonate (Sandoz M.S. 222) in the jar. The strength of the solution was adjusted to produce anaesthesia in about 2 min. In these studies fish were prevented from coming to the surface after sounding by means of a screen cover (Fig. 1). Kokanee and sockeye which have sounded normally commence to refill their swimbladders with atmospheric air within 5 min if they are in quiet, familiar surroundings. Like other salmonids, these fish appear to be incapable of utilizing dissolved gases for swimbladder inflation. Sockeye partly degassed by startling and denied access to the surface failed to show any gain in volume of swimbladder gas over periods of up to 8 days.

DENSITY DETERMINATION

Density of a fish was determined in a series of saltwater baths, the concentrations of which were set by means of oceanographic hydrometers and the tables of Zerbe and Taylor (1953). Salinity increments of 2.5‰ were used over the range of density studied. This corresponds to density differences of approximately 0.002 g per cubic centimeter (cc) between baths. Some further refinement was possible by observing the sinking or buoying of a fish in adjacent baths and interpolating between values. The anaesthetized fish

was transferred from the sounding cylinder to the salt baths and moved up or down the series to the nearest neutral buoyancy. This method of density determination was much more rapid than the various volume–weight methods which have been described, and did not necessitate killing the fish (more than 2000 in this study).

Autonomic Drugs

Several pharmacological agents, with known autonomic effects in the higher vertebrates, were tested in an attempt to establish the nature of the nervous control. Sympathomimetic and parasympathomimetic agents, plus drugs inhibiting or blocking adrenergic and cholinergic responses, were tried. Relatively stable agents, such as ephedrine and pilocarpine, were used in preference to epinephrine and acetylcholine respectively. In practice, the drugs were dissolved in water in 75-liter tanks and tested over a range of concentrations up to the level of initial mortality. After this preliminary screening, the effective dose was used to establish time-response curves.

The drug ephedrine, an effective sympathometic agent, was tested and a time-response curve determined at concentrations of 100 and 200 mg per liter. Time-response curves for "dibenzyline," one of the β-haloalkylamines and a potent adrenergic blocking agent, were established at a concentration of 5 mg per liter. A second, unrelated adrenergic blocking agent "hydergine" (a mixture of three ergot alkaloids) was tested similarly. The effect of cholinergic stimulation on the sounding response was investigated using the parasympathomimetic drug pilocarpine at a concentration of 100 mg per liter. The anticholinergic agents "darbid" (isopropamide iodide) and atropine were tested at doses of 100 and 200 mg per liter respectively.

Swimbladder Volume

The volume of the swimbladder was required for calculation of per cent of gas lost on sounding and for calculation of the release pressure of the pneumatic duct. Calculation of the volume of the bladder is based on the definition of density:

$$\text{density} = \frac{\text{weight}}{\text{volume}}.$$

As the weight of the fish is constant, for all practical purposes, with the swimbladder empty or inflated with gas, density may be related directly to volume (Jones, 1951):

$$D_I \times V_{FI} = D_D \times V_{FD}$$

where D_I is the density of the fish, swimbladder partially inflated; D_D is the density of the fish, swimbladder deflated; V_{FI} is the volume of the fish, swimbladder partially inflated; and V_{FD} is the volume of the fish, swimbladder deflated. But

$$V_{FD} = V_{FI} - V_B$$

where V_B is the volume of the swimbladder at the measured density and volume substituting: $D_D \times (V_{FI} - V_B) = D_I \times V_{FI}$

$$V_B = \frac{V_{FI} \times (D_D - D_I)}{D_D}.$$

The value "deflated density" (D_D) was determined by removing all of the gas from the swimbladder of anaesthetized fish with a syringe and needle and then determining the density. After this the fish were opened midventrally and those in which gas was present in the bladder were discarded from the sample. The deflated density for 60 sockeye smolts was 1.0634 g per cc with ±0.0015 g per cc for one standard deviation, and 1.0551 g per cc with ±0.0040 g per cc for one standard deviation for 16 kokanee. Thus initial density and initial volume of the fish were all that remained to be measured before the volume of the swimbladder could be calculated. For example, where the initial density of the fish was 1.0177 g per cc and the initial volume of the fish was 6.812 ml, the volume of the swimbladder was:

$$V_B = \frac{6.812 \times (1.0634 - 1.0177)}{1.0634}$$

$$V_B = 0.293 \text{ ml.}$$

Swimbladder Pressure

Pressure within the sockeye swimbladder was measured following the method described by Alexander (1959). In this, the expansion of the swimbladder on decompression is compared with the expansion of a similar but unconstrained volume of gas. The pressure to effect the observed constraint is the excess pressure within the bladder.

Duct Release Pressure

The excess pressure within the swimbladder necessary to force gas out through the pneumatic duct was termed the duct-release pressure.

Live fish could not be used in the vacuum-expansion studies, for when exposed to conditions of gradual reduction in pressure, physostomous sockeye simply release gas and return to neutral buoyancy. Individual fish were killed by means of an anaesthetic and placed in a decompression apparatus patterned after that of Alexander (1959). The pressure was reduced from 760 to 45 mm Hg in decrements of 63.5 mm Hg and the volume of the fish recorded. Observations were made on the first evidence of gas loss, size and frequency of the bubbles, and rising and sinking of the fish. The apparatus was calibrated

regularly over the range of decompression and the values obtained for fish were corrected for expansion by the apparatus.

Theoretical expansion of the swimbladder volumes was calculated from the formula for Boyle's Law, and the observed and theoretical swimbladder volumes were plotted as in Fig. 2. Pressure within the swimbladder was calculated from:

$$P_1 \times V_1 = P_2 \times V_2$$

where V_1 is the calculated theoretical volume of the swimbladder; V_2 is the observed volume of the swimbladder at P_1; P_1 is the absolute pressure in mm Hg immediately preceding the release of gas; P_2 is the pressure inside the swimbladder at external pressure P_1. Having derived P_2, the excess pressure within the swimbladder or the net pressure exerted against the duct is simply $P_1 - P_2$. For example, the fish shown in Fig. 2 with a swimbladder volume of 0.293 ml at atmospheric pressure released gas from the swimbladder under conditions of: $V_1 = 0.503$ ml; $V_2 = 0.473$ ml; $P_1 = 442$ mm Hg; $P_2 = \dfrac{0.503 \times 442}{0.473} = 470$ mm Hg. Thus the duct release pressure is approximately 470–442 = 28 mm Hg.

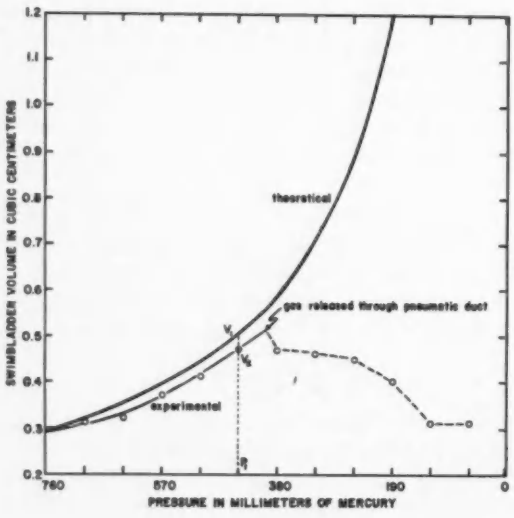

FIG. 2. Calculation of pneumatic duct-release pressure from theoretical and observed expansion of gas in the swimbladder up to time of gas escape.

Evidence was sought for the presence of a sphincter or some form of constriction along the duct. Based on the known tendency of sphincters to relax after the death of an animal, fish were killed and tested after varying periods of time. Drug treatments which had been tested for their effects on the sounding response were retested for their action on the pneumatic duct.

Catheterized Fish

The gulping of atmospheric air (the "Luftschluckenreflex" of Franz) proved more difficult to explore than the loss of swimbladder gas on sounding. The study was simplified by providing the swimbladder with an accessory opening to the exterior of the fish, such that air which entered the swimbladder escaped via the auxiliary duct. This consisted of a catheter of fine polyethylene tubing passing through the side of the fish anterior to the vent and slightly below the lateral line, terminating within the swimbladder. The catheter was secured in place by a small stitch through the adjacent musculature and a tie around the circumference of the fish. Whether or not the catheter ended within the lumen of the swimbladder was apparent from the response of the fish on recovery from the anaesthetic. Properly catheterized fish rose to the surface of the water, mouthed a bubble of air, and angled downward towards the bottom of the aquarium. Concurrent with the change in position from head-up to one of head-down, a series of bubbles, about six in number, issued from the external end of the catheter. This procedure of swimming up to the surface, mouthing a bubble of air, swimming downward and passing the gas into the swimbladder (only to lose it to the outside) was repeated every few minutes for 2 or 3 days. At the end of this time, the fish took to residing on the bottom of the tank and ceased gulping air at the surface.

During the post-operative period when the fish were actively surfacing, gulping air, and losing it through the catheter, the fishes' air-introducing mechanism was amenable to study. In particular, the nervous control of the pneumatic duct was explored by means of drugs in solution. The fish were observed, indirectly, in replicas of the salinity preference chambers of Bagger-man (1957).

Isolated and Intact Swimbladders

The contractile properties of the sockeye swimbladder were investigated in situ in the intact fish and in isolated tissue preparations, of the type routinely employed for the study of isolated smooth muscle. Isolated swimbladders of 18-month-old sockeye were suspended longitudinally within aerated frog Ringer's solution (7–10 C) in a muscle-preparation apparatus and arranged so as to operate a writing lever. Similarly, cross-sectional loops were cut from living swimbladders and suspended in baths as above. These tissue prepararations were tested with adrenergic (adrenaline 10^{-5} and ephedrine 10^{-5}) and cholinergic (acetylcholine 10^{-5} and pilocarpine 10^{-5}) agents. The sympathomimetic drugs were tested both before and after adrenergic blockade was attempted with "dibenzyline" (10^{-5}). By-passing of the "dibenzyline" blockade was attempted with monoacetin at doses 10^{-5}, 10^{-4}, and 10^{-3}, followed by adrenaline (10^{-5}). Similarly cholinergic blockade was investigated with atropine (10^{-5}) followed by pilocarpine (10^{-5}).

The response of the intact swimbladder was investigated using the same pharmacological agents as above. Smolts were anaesthetized, held under

water, and the drugs injected into the body cavity of the fish. Any expulsion of swimbladder gas was obvious from the bubbles emitted from the mouth of the fish. The possible influence of the body wall was explored similarly. Anaesthetized smolts were dissected open midventrally within a bath of physiological saline. With the abdominal walls held apart, a small amount of drug, such as adrenaline, was added to the water in the region of the swimbladder. The response of the swimbladder was noted plus any expulsion of gas from the mouth of the fish.

Histology

The nature of the swimbladder and pneumatic duct was investigated histologically. For this purpose material was fixed in Bouin's picric acid, formolacetic mixture, and prepared routinely with paraffin embedding and hematoxylin-eosin staining.

RESULTS

Gas Loss on Sounding

Sixty sockeye smolts held 2 days in water 50 cm deep showed a mean density of 0.9995 g per cc with ±0.0009 for one standard deviation. Sockeye smolts induced to sound in a glass container showed a mean density after sounding of 1.0244 g per cc with ±0.090 for one standard deviation. This was an average loss of 38% of gas from the swimbladder. The most extreme loss shown by an individual fish was 72% of its swimbladder gas on sounding In the drug-evaluation studies, 720 control sockeye sounded to a mean density of 1.0214 g per cc, an average gas loss of 34%. Of the kokanee yearlings, 120 sounded to a density of 1.0120 g per cc, with ±0.0060 for one standard deviation. This was an average gas loss of 23%.

Sockeye salmon smolts, held in a solution of ephedrine, evidenced the same sounding response as control fish when tested. However, significantly more gas was lost from the swimbladder on sounding. Two series of 10 fish, each exposed to 100 mg per liter of ephedrine, sounded to a mean density of 1.0280 g per cc, representing a gas loss of 44% (Fig. 3). Ephedrine-treated and control fish differed significantly with t-test values of 3.93 and 2.92 ($t_{0.005}$ = 2.915). Smolts held in 200 mg per liter of ephedrine before testing differed even more from the control fish on sounding with t values of 5.60 and 7.22 for the two series. Kokanee exposed to 100 mg per liter of ephedrine sounded to a mean density of 1.0186 (Fig. 4) for an average gas loss of 35%. Ephedrine-treated kokanee differed significantly from controls (t = 8.33, 7.32 for two series).

In the first "dibenzyline" series, the test and control groups of sockeye did not differ significantly during the first 2 hr, with t = 0.67 ($t_{0.5}$ = 0.679) but did differ significantly thereafter. In the second series, gas loss was reduced even in the 1- and 2-hr treated groups. The combined results appear in Fig. 5. Kokanee exposed to 5 mg per liter of "dibenzyline" showed a maximum

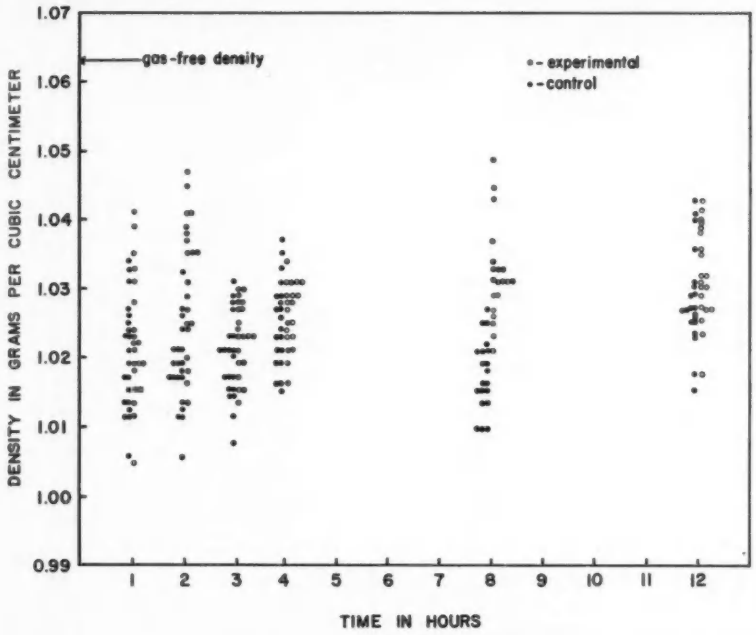

FIG. 3. Density of sockeye smolts after sounding. Test fish exposed to 100 mg per liter of ephedrine for up to 12 hr.

difference from controls after 1 hr of treatment (t = 5.12; $t_{0.001}$ = 3.460) for the combined series (Fig. 6). At every tested interval some treated kokanee continued to release a small amount of gas but the majority ceased expelling gas. The amount of gas expelled by the kokanee was least at 12 hr with an average density of 1.0020 g per cc. The treated sockeye stopped blowing gas completely after 4 hr exposure to "dibenzyline" for an average density of 0.9993 g per cc. The sounding response was unaltered but for failure of gas loss from the swimbladder. In both sockeye and kokanee a marked chromatophore response was apparent after 1–2 hr exposure to "dibenzyline."

Fish treated with 10 mg per liter of the adrenergic blocking agent "hydergine" also showed significantly less gas loss on sounding than the control fish (t = 3.87; $t_{0.001}$ = 3.460).

Cholinergic stimulation and inhibition yielded less clear results than the adrenergic studies described above. Fish held in a solution of 100 mg per liter of pilocarpine lost significantly more gas on sounding than the control fish. The cholinergic blocking agent "darbid" did not alter gas loss on sounding, but fish treated with atropine (200 mg per liter) showed a significant reduction.

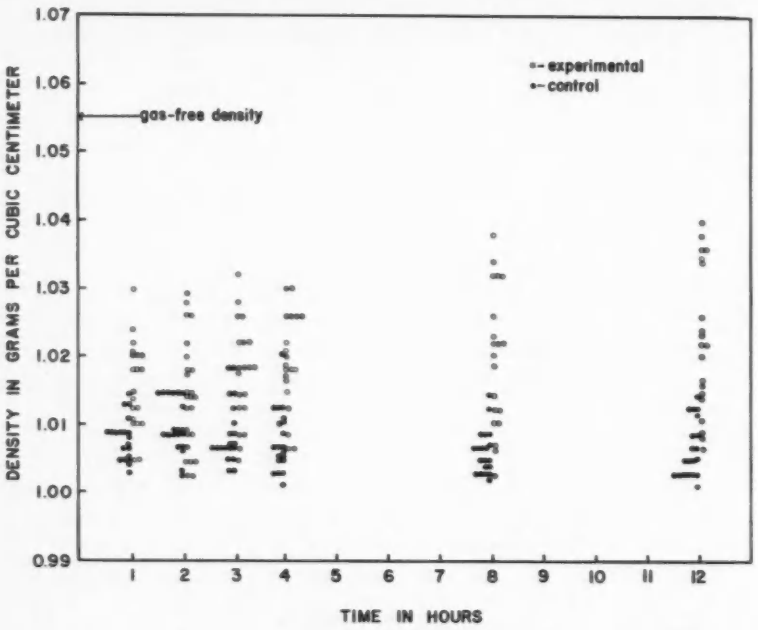

FIG. 4. Density of kokanee yearlings after sounding. Test fish exposed to 100 mg per liter of ephedrine for up to 12 hr.

SWIMBLADDER AND DUCT-RELEASE PRESSURE

Ten sockeye yearlings yielded an average excess pressure of 0.2 mm Hg, with ±5.5 mm Hg for one standard deviation. For so low an excess pressure within the swimbladder, there was little or no separation of the curves of gas expansion. That is, the expansion curve for gas confined within the swimbladder and the curve for that gas outside the swimbladder were very nearly identical. Nevertheless, the method was sufficiently precise to show that the pressure within the sockeye swimbladder did not appreciably exceed the external pressure on the fish.

In the study of duct-release pressure, three control series of 10 fish each showed average release pressures of 28.9, 28.6, and 26.9 mm Hg. No significant difference was found among the three control series ($P = 0.5$). Fish tested at intervals after death showed a progressive reduction in duct-release pressure with a regression line of $Y = 27.18–0.78X$. Fish tested over the period of 5–30 min after death differed significantly from control fish at time zero, with $t = 4.15$ ($t_{0.005} = 3.397$). Beyond 30 min of holding after death, duct-release pressure increased rapidly and in three fish exceeded that of the controls. This coincided with the onset of rigor.

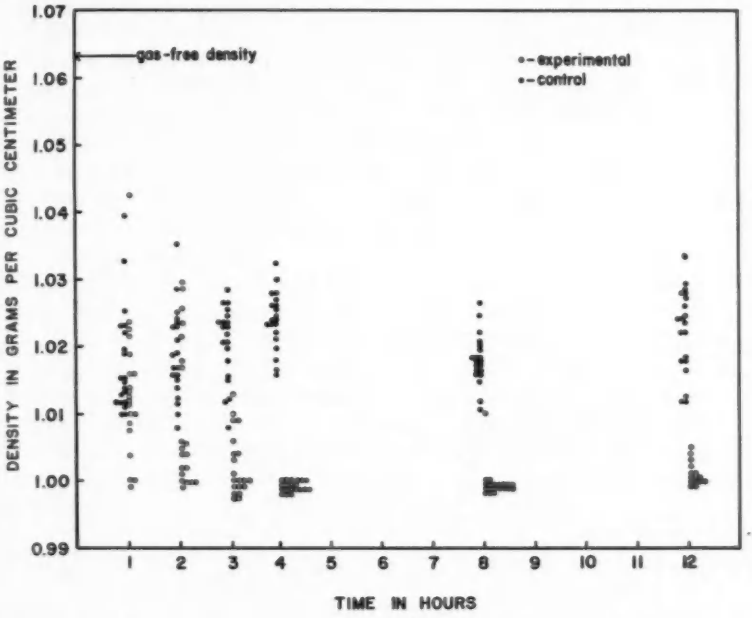

FIG. 5. Density of sockeye smolts after sounding. Test fish exposed to 5 mg per liter of "dibenzyline" for up to 12 hr.

"Dibenzyline"-treated fish showed a mean duct-release pressure of 26.8 mm Hg and were not significantly different from control fish ($P = 0.5$) Atropine-treated fish showed a mean duct-release pressure of 0.2 mm Hg and differed significantly from control and "dibenzyline" fish with $t = 5.32$ ($t_{0.001} = 4.781$).

CATHETERIZED FISH

Smolts, catheterized and then held in "dibenzyline" solution (4 hr at 5 mg per liter), continued to surface, mouth air, and pass gas into the swimbladder. There was thus no evidence that this drug was influencing the ability of the fish to inflate their swimbladders with atmospheric air. This was confirmed on noncatheterized fish. These were induced to sound, occluded from the water surface by means of a screen, and in this partly de-gassed condition exposed to "dibenzyline" for 4 hr. After removal of the screen at the end of this time, treated and control fish promptly returned to the surface and inflated their swimbladders to neutral buoyancy.

Catheterized smolts treated with atropine similarly showed continued attempts at swimbladder inflation. As was the case with "dibenzyline," atropine

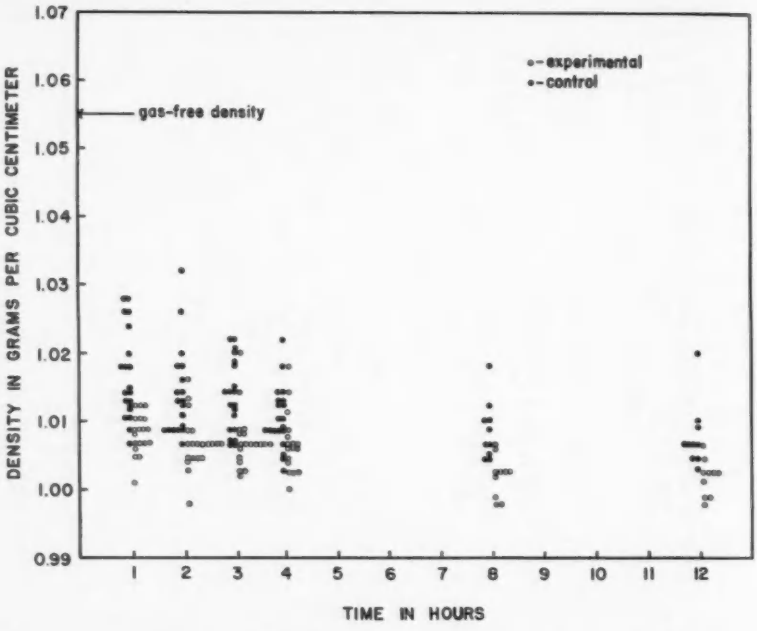

FIG. 6. Density of kokanee yearlings after sounding. Test fish exposed to 5 mg per liter of "dibenzyline" for up to 12 hr.

did not interfere with the process of air passing into the swimbladder. The response, however, was slower in the atropine-treated fish as compared with the controls.

ISOLATED AND INTACT SWIMBLADDERS

Isolated swimbladders, suspended longitudinally in Ringer's solution, showed little or no reduction in length when exposed to adrenaline or acetylcholine (10^{-5}). Cross-sectional loops, similarly tested, evidenced a marked reduction in length when tested with adrenaline and ephedrine (10^{-5}). Such loops exposed to "dibenzyline" (10^{-5}) evidenced a gradual relaxation and could not be stimulated to contract with adrenaline or ephedrine. The "dibenzyline" blockade could not be by-passed with monoacetin over the range of concentration of 10^{-5} to 10^{-3}. Adrenaline-induced contraction and "dibenzyline" relaxation were relatively slow in isolated-loop studies. These effects lasted 3–5 min, in contrast to gas loss on sounding which is usually complete in 30 sec. Little or no response accompanied treatment of isolated loops with acetylcholine, pilocarpine, or atropine at a concentration of 10^{-5}.

HISTOLOGY

Macroscopically, the pneumatic duct of the kokanee and sockeye was found to be a convoluted tubule joining the oesophagus and swimbladder. The duct was continuous with the dorsal surface of the oesophagus (Fig. 7A) but turned laterally (Fig. 7B, C) before it joined the anterior end of the swimbladder (Fig. 7D). The lateral convolutions of the pneumatic duct make it impossible to trace the lumen from oesophagus to swimbladder in any single, histological section. No "pneumatic bulb" or oesophageal bulb was found in sockeye or kokanee salmon, such as has been reported in cyprinids (Evans and Damant, 1928).

Microscopically, the pneumatic duct consisted of a mucosa and submucosa. The mucosa was made up of three layers: an epithelium of tall columnar cells; a tunica propria of dense connective tissue; a muscularis mucosae of circular and some longitudinal muscle fibers (Fig. 7A, C). The submucosa of loose (areolar) connective tissue was more apparent in the longitudinal sections (Fig. 7B, D) than in the cross-section (Fig. 7C) of the pneumatic duct of the kokanee illustrated. The swimbladder evidenced the same structure but the longitudinal muscle layer was much reduced or absent (Fig. 7D).

The pneumatic duct was surrounded by smooth muscle fibers arranged circularly and any part of this duct may be capable of constriction. In addition, striated muscle fibers were present at the point where the duct enters the wall of the oesophagus (Fig. 7A). In yearling sockeye and kokanee, the striated fibers were in the form of strands within the muscularis; in adult fish (Fig. 8) the striated muscle formed a sphincter within the muscularis mucosae, encircling the duct at the point where it penetrated the muscularis of the oesophagus.

DISCUSSION

When frightened, sockeye and kokanee salmon dive into deeper water and this escape behaviour is accompanied by loss of gas from the swimbladder. This increase in density in response to fright has been observed previously for various fishes. Kuiper (1915) found that expulsion of gas from the swimbladder of physostomes could be induced by illumination, vibrations in the water, chemicals, and enclosing fish in a confined space. Dijkgraaf (1932) observed that alarmed minnows expelled gas from their swimbladder and this response was associated with sound production. Franz (1937), in his study of decompression and the "Gasspuckreflex" noted that minnows moved to another container frequently responded by spontaneous gas "spitting." Plattner (1941) confirmed Kuiper's observations. He observed *Phoxinus* to spit bubbles of gas and seek the bottom of their container when disturbed. Similarly, fish stimulated by a flashing light, or by striking the container, ejected bubbles from the mouth. Fänge (1953) noted the similarity between the deflatory reflex in physoclists and the Gasspuckreflex in physostomes.

He suggested the deflatory reflex facilitated the escape of fish into deeper water through a decrease in swimbladder volume. Verheijen (1962) observed gas spitting in response to visual and mechanical disturbance among white suckers, creek chubs, and bluntnose minnows, and in response to alarm substance in the water among hornyhead chubs.

Kokanee and sockeye salmon were observed to emit gas and swim downward in response to the same stimuli: light, mechanical disturbance, or sudden change in visual field. Although all kokanee and sockeye induced to sound lost some gas from the swimbladder, the amount of gas lost varied with the nature and intensity of the stimulus. Sockeye induced to sound by a disturbance on the water surface lost an average of 23% of bladder gas. Sockeye placed in the illuminated glass cylinder lost 34% and kokanee 23% of swimbladder gas.

Pneumatic Duct Control

The control of gas loss from the physostomous swimbladder was investigated first by Guyénot (1909). He found that in cyprinids the pneumatic duct was provided with a sphincter, the tonicity of which maintained gas in the bladder. Histological study of the sockeye and kokanee swimbladders and pneumatic ducts evidenced some striated muscle fibers surrounding the pneumatic duct at the point where it enters the wall of the oesophagus. In yearling, fish, the striated muscle was in the form of strands of a few fibers near the duct. In adult fish, a large band of striated muscle, triangular in cross-section, circled the opening of the duct. The band of striated muscle was not so discrete as that described for the rainbow trout by Weinreb and Bilstad (1955).

In young sockeye, the fact that the pneumatic duct was exerting a sphincter-like control is apparent from the excess bladder pressure necessary to force gas out through the pneumatic duct. For 30 fish, an increase in pressure from the normal bladder pressure of 0.2–28.1 mm Hg was necessary to force gas out of the swimbladder. After the death of the fish, this pressure at release declined rapidly to 5 mm Hg.

Both Kuiper (1916) and Plattner (1941) concluded that the pneumatic duct sphincter was innervated antagonistically; stimulation of the vagus in cyprinids caused relaxation of the sphincter with release of gas, whereas anaesthesia and sectioning of the vagus retarded opening of the sphincter. Normally the sphincter was relaxed, as evidenced by the amount of decompression required to induce gas loss. Fänge (1953) noted the control of the pneumatic duct, as described by Kuiper and Plattner, did not agree with the sympathetic control of the deflatory reflex in physoclists. Fänge observed the pneumatic sphincter to constrict in *Salmo irideus* in response to acetylcholine. This observation agrees with the effect of atropine in reducing to almost nil (0.2 mm Hg) the duct-release pressure in sockeye. Fänge raised the possibility that the deflatory reflex is conducted by sympathetic fibers running in the vagus.

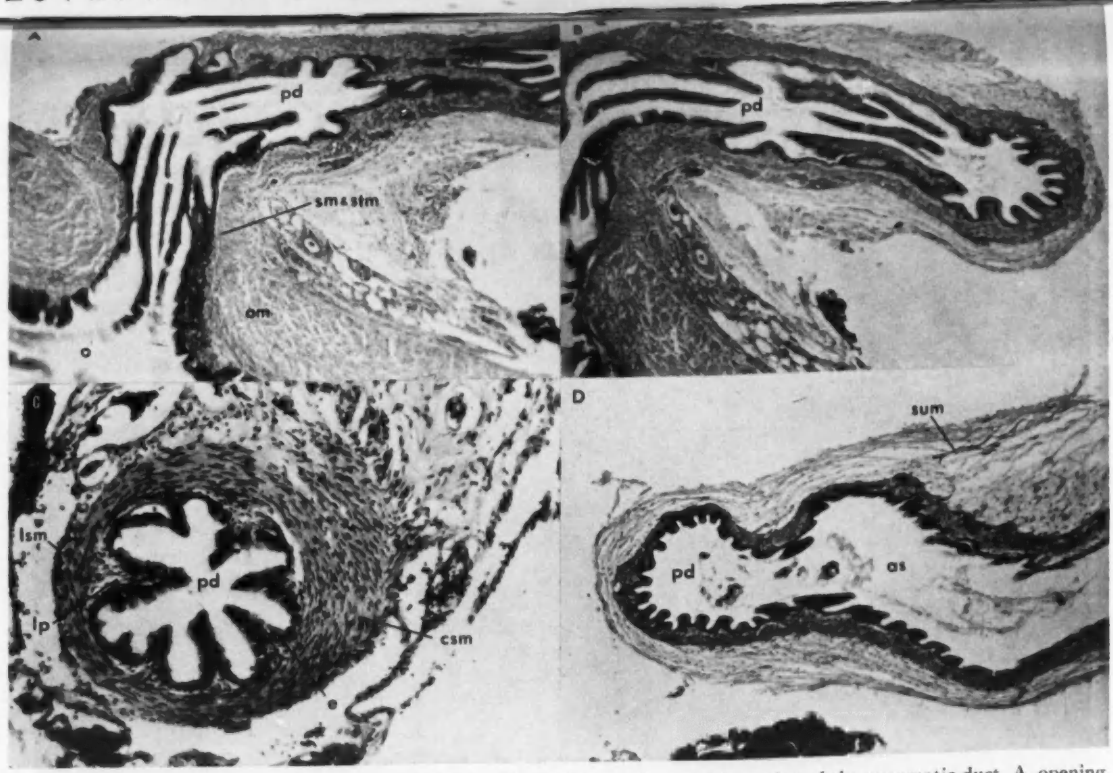

FIG. 7. Photomicrographs of sagittal sections of a yearling kokanee salmon in the region of the pneumatic duct. A, opening of the pneumatic duct on the dorsal surface of the oesophagus; B, pneumatic duct between oesophagus and swimbladder; C, pneumatic duct where it runs laterally dorsal to the oesophagus; D,, pneumatic duct enters obliquely into the anterior swimbladder. *o* = oesophagus; *om* = oesophageal musculature; *pd* = pneumatic duct; *sm* = smooth muscle; *stm* = striated muscle; *lp* = lamina propria; *lsm* = longitudinal smooth muscle; *csm* = circular smooth muscle; *e* = epithelium; *as* = anterior swimbladder; *sum* = submucosa. Magnification A, B, D, 55×; C, 160×.

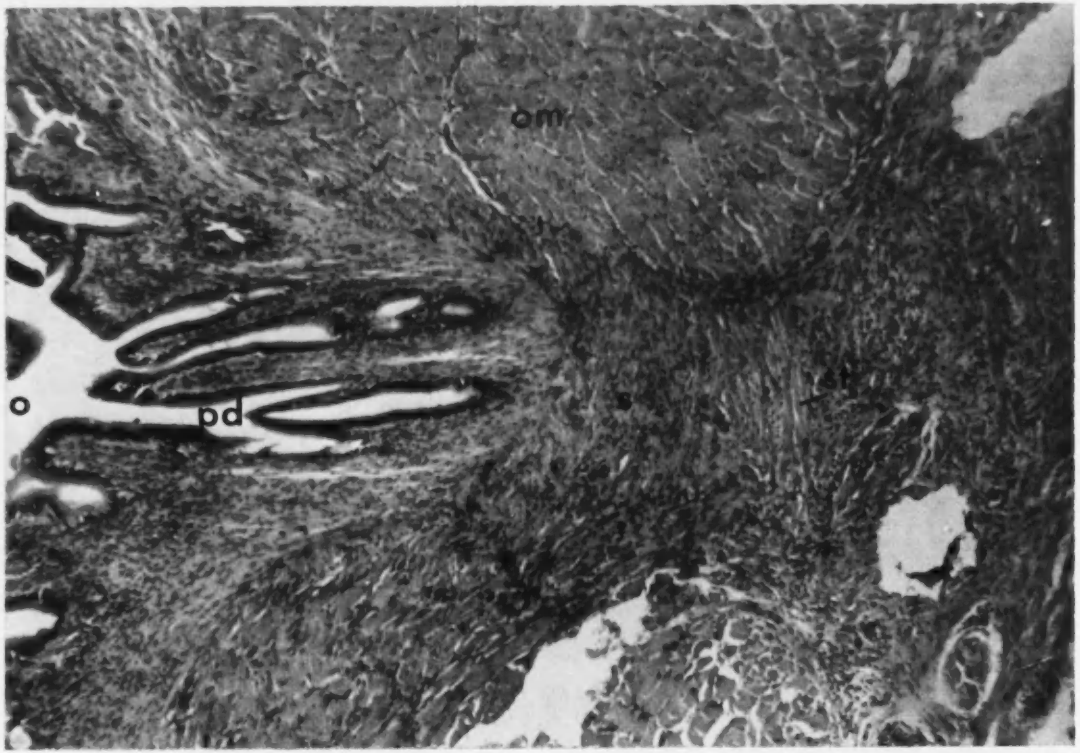

FIG. 8. Photomicrograph of the pneumatic duct region of an adult kokanee salmon. The section illustrated was cut slightly out of the plane of the lumen to show the smooth and striated muscle. Examination of serial sections revealed these smooth and striated fibers encircled the duct in this region. *o* = oesophagus; *om* = oesophageal musculature; *pd* pneumatic duct; *s* = smooth muscle; *st* = striated muscle. Magnification 100×.

Adrenergic blockade with "dibenzyline," although it prevented gas loss from the swimbladder, did not change significantly the duct-release pressure. Ten "dibenzyline"-treated fish showed an average duct-release pressure of 24.2 mm Hg. Nor did blockade with atropine or "dibenzyline" interfere with fish gulping atmospheric air at the surface and inflating previously emptied swimbladders. These results would suggest that, in the kokanee and sockeye, the duct mechanism is under cholinergic control and is not necessarily involved directly in gas expulsion.

Swimbladder Contractibility

The presence of an extensive layer of circular muscle fibers in the swimbladder of the brown trout was shown by Corning (1888) and Rauther (1922) and for the rainbow trout by Eissele (1922). In young kokanee and sockeye salmon, the layer of circular muscle fibers dominated the muscularis mucosae; the longitudinal fibers forming a layer only a few cells thick.

Franz (1937) noted the musculature of the swimbladder and suggested three possible functions: (1) changes in volume facilitating swimming movements; (2) maintenance of excess bladder pressure; (3) emission of gas from the swimbladder.

He tested these possible functions experimentally, confirming the first and refuting the second. As for the third function, Franz decompressed certain cyprinids to 120 mm Hg absolute, during which the fish released gas from the swimbladder. He then restored these fish to neutral buoyancy by injecting gas into the body cavity. Fish so treated were still capable of expelling gas from the swimbladder. From this Franz concluded gas expulsion must be due to contraction of bladder musculature and could not result from excess pressure within the bladder. It is surprising that fish with swimbladders less than one-sixth inflated were still capable of expelling gas. In the case of sockeye and kokanee, frightened fish were incapable of expelling the last one-quarter to one-third of the gas in the bladder. Jones and Marshall (1953) suggest gas may be ejected from the cyprinid swimbladder through the pressure of the gas (60–80 mm Hg). In sockeye and kokanee, the pressure of gas in the bladder is very low, averaging 0.2 mm Hg. This result, plus the amount of gas lost from the swimbladder (up to 72%) argues for expulsion as a consequence of bladder contraction.

Fänge (1953) also noted that expulsion of gas from the swimbladder of physostomes involved contraction of the smooth muscle layer of the swimbladder. He suggested that this muscularis mucosae is probably innervated by adrenergic fibers.

Isolated swimbladders of sockeye, suspended longitudinally in Ringer's solution and exposed to adrenaline and to acetylcholine, evidenced little or no reduction in length. However, cross-sectional loops from the sockeye swimbladder, exposed to the sympathomimetics adrenaline and ephedrine, showed a marked reduction in length. Such loops exposed to the adrenergic blocking agent "dibenzyline" were induced to relax and could not be stimulated to

contract by further treatment with adrenaline or ephedrine. Injection of adrenaline into the body cavity in the region of the swimbladder caused sockeye to expel gas underwater.

These experiments argue strongly for the loss of gas in sockeye and kokanee being due to contraction of circular muscles in the bladder wall, and the stimulation of these muscles being under adrenergic control.

Significance of the Sounding Response

Sockeye and kokanee salmon are pelagic fishes of the lacustrine environment for part or all of their life. The adult fish spawn in streams inlet to or outlet from a lake, or in the shallow water of the lake itself. In early spring, emerging fry typically move downstream into the lake environment. The fry remain only briefly inshore before occupying the pelagic zone of the lake. Fingerling and yearling sockeye are most abundant in surface waters in spring but are forced downward with rising epilimnial temperatures during thermal stratification (Ward, 1932). Similarly, kokanee are distributed in the region of the thermocline during the day, feeding near the surface at night (Lorz, 1962). During winter isothermal conditions, young sockeye are distributed almost uniformly to a depth of 50 m (Krogius and Krokhin, 1948).

Kokanee and sockeye salmon are schooling fishes. Sockeye form schools on emerging from the gravel (Hoar, 1954), and this schooling behavior continues throughout the life of the animal until the mature fish occupy territory on the spawning grounds. In nature, schools may be large and contain hundreds or thousands of animals. In small experimental schools, the sounding response of a single individual may be sufficient to induce the school to sound.

Young sockeye in Cultus Lake are the object of intense predation (Foerster, 1938); their predators are squawfish, trout, and char. For young sockeye living in the region of the thermocline, the sounding response would carry these fish into the poorer light of the hypolimnion and thus may give some protection from predators. If the predator takes the hindmost from a school of sounding kokanee or sockeye, then selection would favor fish best able to swim downward. Sounding would be facilitated by sudden density increase, such as occurs with expulsion of swimbladder gas. Gas loss on sounding may thus be viewed as an adaptation offering intraspecific advantage among sockeye and kokanee salmon. The increase in downward swimming that accrues from this sudden density increase has yet to be quantified. This is being investigated currently.

Acknowledgments

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Daily Feeding Periodicities, Food Uptake Rates, and Dietary Changes with Hour of Day in Some Lake Fishes[1]

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ABSTRACT

The feeding periodicities of five common cohabiting fish species in Lake Opinicon, Ontario, were defined for the early summer period of accelerated feeding by determining the mean weights of stomach contents per gram of body weight at intervals of 2–3 hr throughout the 24-hr cycle. Two, and in sunfish three, feeding peaks per 24-hr period were indicated. *Perca flavescens* was diurnal; *Lepomis macrochirus*, *L. gibbosus*, and *Fundulus diaphanus* were largely diurnal (with a nocturnal component); and *Ambloplites rupestris* was equally nocturnal and diurnal. In the two sunfish there were clearcut differences in the organisms eaten by day and night. The technique developed here is a useful method for determining the mean minimum daily ration for a population of fish in the field. The results are comparable with those obtained by other workers in aquarium feeding experiments.

INTRODUCTION

Although it is known that fish species may differ widely in their daily patterns of feeding activity (e.g. compare results of Spencer, 1929; Spoor and Schloemer, 1938; Darnell and Meierotto, 1962), these patterns have been defined for only a few species. The ecological significance of such differences in relation to kinds of food eaten and interspecific competition has received little attention.

The feeding periodicities for five cohabiting species of lake fishes are discussed here, on the basis of mean weights of stomach contents per gram of body weight for different times during the 24-hr period. The findings are used to develop a mean quantitative figure for the amount of food eaten in a day. Data are also given on differences in the kinds of foods consumed at different times of day.

METHODS

The study was carried out in Lake Opinicon, Leeds County, Ontario, and is based on two 48-hr periods of netting on June 1–2 and June 15–16, 1966, that is, during the period of accelerated early summer feeding. Water temperatures at the time ranged from 18 to 22 C.

Fish were netted at two sites: in the shallow waters (2–5 ft) in Birch (Curtis) Bay, using seines, and from the foreshore adjacent to the Queen's University boathouse out towards the middle of the lake (water depth 6–15 ft) by means of gillnets. The seining was carried out at 3-hr intervals and

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the gillnets were cleared every 2–2½ hr. This gave samples at intervals of 1½–3½hr, except in a few instances where none, or only a few individuals of a particular species, was caught. None of the fish taken by seine vomited food after collection nor, as far as could be determined, did those gillnetted, the weights of their stomach contents at the various times paralleling those obtained by seine.

The fish were killed, slit, and preserved in 10% formalin immediately after collection. The total length, body weight, and weight of stomach contents for each individual were taken. The weighing was done after removal of superficial fluid, i.e. so as to approximate the natural consistencies of both fish and food organisms. Weight of stomach contents per gram of body weight was derived for each fish. From these a mean was calculated for each species for each sampling period (Fig. 1–5).

The total lengths and weights of the species were: banded killifish (*Fundulus diaphanus*, Cyprinodontidae), 60–85 mm, 2.5–5.4 g; rock bass (*Ambloplites rupestris*, Centrarchidae), 100–170 mm, 29.0–90.0 g; pumpkinseed (*Lepomis gibbosus*, Centrarchidae), 100–140 mm, 18.0–62.0 g; bluegill (*L. macrochirus*, Centrarchidae), 90–140 mm, 11.1–58.0 g; and yellow perch (*Perca flavescens*, Percidae), 90–130 mm, 7.8–26.5 g.

Since fish of different body sizes vary in their food uptake rates, samples of each species were restricted to the commonest and most convenient size-class. Broadly speaking, the growth rate for percoids in Lake Opinicon appears to be slightly faster than that described for Michigan by Beckman (1949). Hence, the yellow perch were between 1 and 2 years of age, the pumpkinseeds and bluegills between 2 and 3 years, and the rock bass between 2 and 4 years of age. *Fundulus diaphanus* was more than 1 year old and possibly between 2 and 3 years. None of the species had reached their maximum size other than *F. diaphanus*.

Lake Opinicon, 6 × 1 miles, has a surface area of 2200 acres. Its maximum and mean depths are 35 and 15 ft respectively, and approximately half the area is less than 15 ft in depth. The shoreline is sloping and only in a few areas (e.g. Birch Bay) do extensive areas of unimpeded shallows (i.e. up to 5 ft in depth) permit seine netting. The lake is mesotrophic. Chemical analyses carried out by the National Health and Welfare Laboratory, Kingston, Ont., in 1966, gave the following results: total dissolved solids, 120 ppm (July) and 129 ppm (September); *p*H, 8.2 (July), and 8.7 (September); total alkalinity $CaCO_3$, 76 ppm; total hardness, 97 ppm (D. Sheppard, unpublished MS).

Twenty-two species of fish have been recorded for the lake but only 12 of these occur regularly or are numerous. The five species treated here are the common ones over most of the lake. Bluegills are the most numerous. Killifish are largely confined to sandy shallows (e.g. Birch Bay). The foods of Birch Bay fishes have been discussed in Keast (1965) and the relationship of morphology to food niche in Keast and Webb (1966).

FEEDING PERIODS OF THE SPECIES

Yellow perch.— There were two peaks in weight of stomach contents per gram of fish (Fig. 1), a high one at dusk (about 8 PM) and a lower one in mid-morning (about 10 AM). The periods of rapid rise, which indicate the

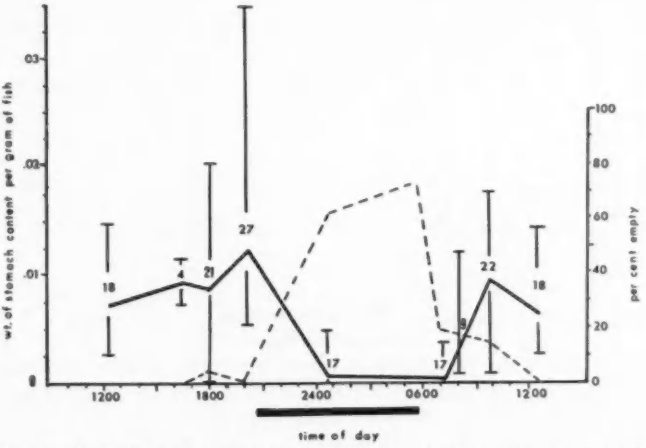

FIG. 1. Daily feeding periodicity in yellow perch (*Perca flavescens*). Solid curve, mean weights (g) of stomach contents per gram of fish; broken line, percentages of stomachs empty. Vertical lines above means, highest weights of stomach contents per gram of fish; below means, lowest. Numerals, numbers of fish in samples (total 148). Solid bar, hours of darkness. Data obtained over two 48-hr periods, June 1–2 and 15–16.

start of active feeding, began at about 6 PM and 7 AM, respectively. The trends in values for individuals with the highest weights of contents per gram of fish and for the means were similar. The curve for percentage of fish with empty stomachs was in general the reciprocal of the feeding curve. The range for stomach content weights was great at all times.

In Lake Mendota, Wisconsin, yellow perch show pronounced migratory movements towards sunset, congregating in certain well-defined feeding areas; there is a less clearcut migration at dawn (Hasler and Bardach, 1949).

Rock bass.— There were two peaks in weight of stomach contents per gram of fish, one extending from about 9 PM to 1:30 AM and the second about noon (Fig. 2). Active feeding commenced about 5 PM (4 hr before dark), and stomachs reached their maximum fulness about 9 PM. Thereafter they remained nearly full until about 1 AM, after which they emptied rapidly. A period of morning feeding started about 7:30 AM, a couple of hours after dawn. The values for the individuals with the fullest stomach for the various sampling periods, and for percentages of fish with empty stomachs, supported this feeding cycle. Again a marked variation in the degree of stomach fulness at any time was apparent.

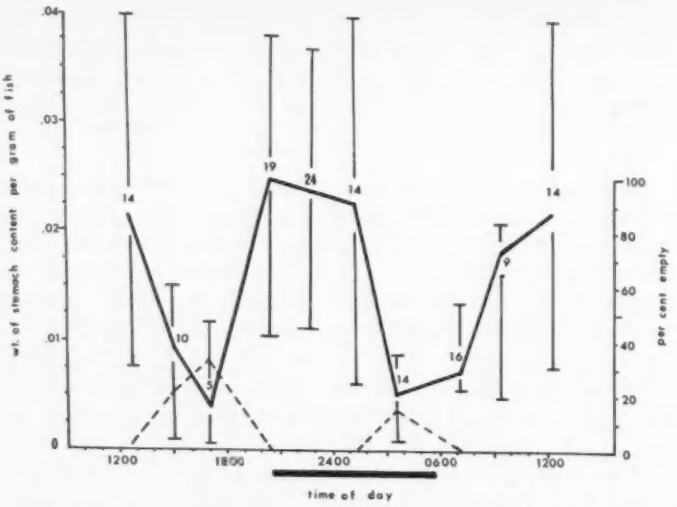

FIG. 2. Daily feeding periodicity in rock bass (*Ambloplites rupestris*). Details as in Fig. 1. Total fish 125.

Activity in the rock bass in Muskellunge Lake, Wisconsin, peaks between 7 and 9 PM; the fish remain relatively active throughout the night, and show a slight increase in activity between 3 and 4 AM (Spoor and Schloemer, 1938).

Pumpkinseed.— There were three peaks in degree of stomach fullness: high ones at about 6 PM and 8 AM, and a lower, intermediate one at about 3 AM (Fig. 3). The pumpkinseed is, hence, largely diurnal in its feeding. Accelerated feeding started at about 5 AM, and continued until about 7:30 AM. Thereafter stomachs emptied until about 9:30 AM when steady feeding resumed. This was followed by a marked acceleration between 3 PM and 5:30 PM. The stomachs then emptied steadily until midnight, after which there was a brief period of feeding and then an emptying until dawn. Variation in the degree of stomach fulness was marked, there always being some fish with empty stomachs.

Bluegill.— Two main periods of feeding are suggested for the bluegill (Fig. 4). The afternoon peak occurred at 3 PM, earlier than in the pumpkinseed. Stomachs progressively emptied thereafter until about 8:30 PM, when feeding recommenced. Intermittent feeding occurred during the night but by 7:30 AM 20% of the fish had empty stomachs.

That the bluegill is a near continuous feeder at this time of the year is indicated by no more than 20% of the fish having empty stomachs at any time and by the weight of stomach contents per gram of fish never falling below the 0.004 level. This contrasts with *L. gibbosus*, in which the stomachs

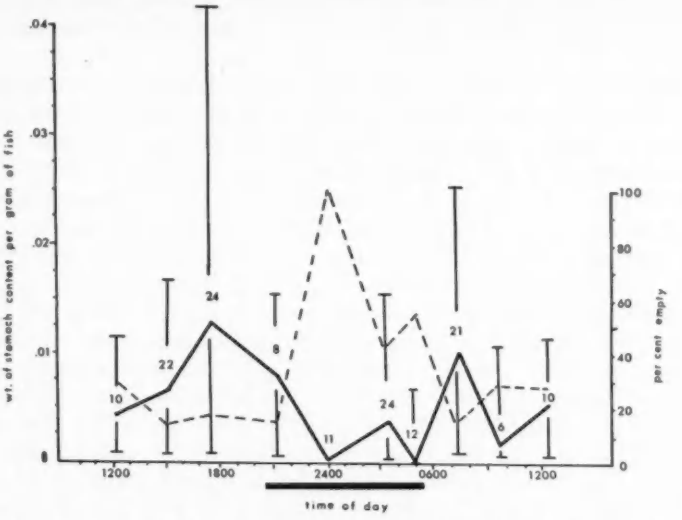

FIG. 3. Daily feeding periodicity in pumpkinseed (*Lepomis gibbosus*). Details as in Fig. 1. Total fish 148.

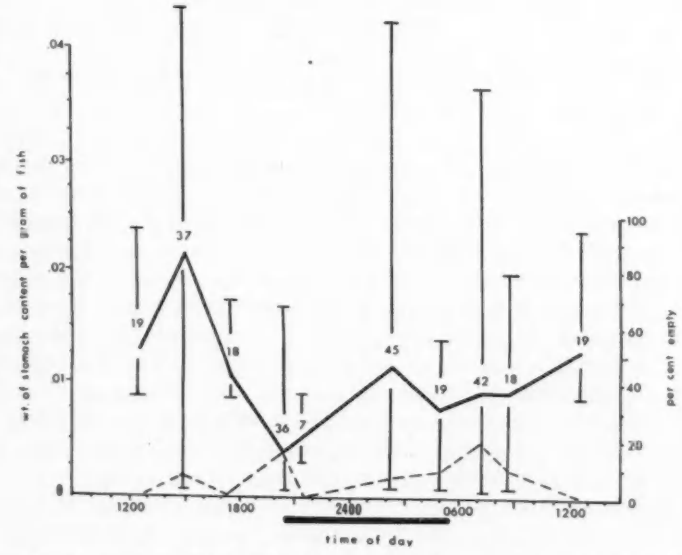

FIG. 4. Daily feeding periodicity in bluegill (*Lepomis macrochirus*). Details as in Fig. 1. Total fish 271.

emptied completely in the middle of the night. Seaburg and Moyle (1964) record that, in Minnesota, bluegills feed "fairly regularly throughout the day and night."

Banded killifish.— This species was largely diurnal in its feeding, most food being obtained between 1 and 5:30 PM (Fig. 5). Thereafter, the stomachs emptied until about 1 AM when 70% of the fish contained no food. A second, lesser period of feeding commenced before dawn. By 10 AM 65% of the stomachs were empty.

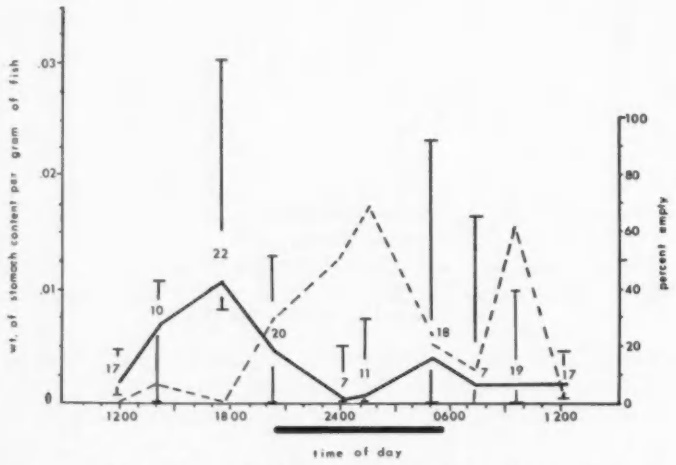

FIG. 5. Daily feeding periodicity in banded killifish (*Fundulus diaphanus*). Details as in Fig. 1. Total fish 131.

Comparison of the species.— It is clear from the above that the feeding cycles of the different species differed appreciably. The yellow perch is diurnal and has feeding peaks in late afternoon and in the morning. The pumpkinseed, bluegill, and killifish have a major peak in the afternoon, and a minor one in the morning. Both sunfishes did some feeding in the middle of the night. The rock bass fed from 5 PM to about midnight, and from about 7:30 AM to noon. The brown bullhead (*Ictalurus nebulosus*), the common species in the muddier areas of the lake which sometimes enters the areas studied, is apparently exclusively nocturnal in its feeding. This is well documented by Darnell and Meierotto (1962) for Wisconsin. In Lake Opinicon only fish collected at dawn and in the very early morning have full stomachs: those taken later in the day or in the early part of the night are empty or near empty.

The species differences above suggest that two different stimuli may initiate feeding: a lowering of the food level in the stomach in the case of the sunfish, and a photoperiodic effect in the case of more cyclic feeders such as the yellow perch (and brown bullhead).

FEEDING RATES

Figures 1–5 bring out clearly that at any peak of stomach fullness the mean value for the population is considerably lower than that for the individual with the fullest stomach. This latter figure is fairly constant for the various species, ranging from 0.030 to 0.045 g per gram body weight. Since the mean levels are only half this, or less, it is obvious that the "average" stomach is far from filled to its potential during a feeding period. There may be various reasons for this. An individual that "over-eats" during one feeding period may consume correspondingly less at the next one. Alternatively, metabolic needs may adequately be met by less than the maximum intake of food, feeding may be rhythmic, or the amount of food that can be obtained in a given time might simply be limited. The lengths of time between feeding peaks agree with published data on digestive rates of centrarchids at this temperature range. Thus, Seaburg and Moyle (1964) found that in bluegills under near-natural conditions at 18–23 C 50% of the stomach volume was digested in 5 hr, and 75% in 12 hr. Windell (1966) calculated figures for force-fed bluegills at 21 C as follows: 50% digestion in 6 hr, and 100% digestion in 18 hr. A voluntary meal, however, was 85% digested in 10 hr.

The cyclical nature of the feeding in these fish, and the clearcut development of peaks, provide a basis for calculating the daily ration in the Lake Opinicon fish. This was done from the graphs by determining the differences between the mean weights of stomach contents at the peaks and the values for the succeeding low points (troughs). The resultant figures are: yellow perch, 0.020 g (0.011, and 0.009 added) per g of body weight; rock bass, 0.04; pumpkinseed, 0.026; bluegill, 0.025; and killifish, 0.014. The method is satisfactory in cases where the stomach is emptied between feeding periods, least so (as in bluegills) where feeding is erratic or near-continuous. At the same time since some digestion must take place during feeding periods (i.e. from the preceding low to the high) and, in some species, there may be feeding when the volumes are lessening, the calculations are probably minimum values.

The finding here that the fish (other than the killifish) consumed about 2–4% of body weight daily agrees with data from other sources. Thus hatchery brook trout, held at 16 C and fed a ration designed to give maximum food conversion and growth, consume 6% of the body weight daily when 4–5 inches long, and 2% when 9–10 inches long (Third (1952) Edition of the New York State Hatchery Feeding Chart). Gerking (1954) found that a daily ration of 3% was close to maximum in aquaria bluegills fed mealworms and maintained at room temperature. Seaburg and Moyle (1964) estimated a daily ration for lake bluegills, pumpkinseed, and crappie in Minnesota as 1–2% of body weight. This figure, however, represents the average for the whole summer, not the time of accelerated spring feeding as in the Lake Opinicon fish. Seaburg and Moyle calculated the average daily meal on the basis of the "product of the average stomach volume for the summer, the percentage of food found experimentally to be digested in 4 hours, and the number of such 4-hour digestion periods in a day."

The mean figure for killifish, the smallest-bodied species, was unusually low. Further study is needed here. It may, however, have been purely a seasonal effect since the individuals studied, besides being of maximum body size (and hence presumably with a somewhat lower metabolism than the other species), were about to breed. The values for rock bass were, by contrast, high. This species, a "macropredator," has a very large sac-like stomach. The values may be inflated by a preponderance of large, heavy prey items (crayfish, Anisoptera nymphs, and fish) which tend to clear the stomach only slowly.

CHANGES IN KINDS OF FOOD EATEN DURING THE 24-HR PERIOD

Yellow perch.— The foods consumed were similar during the two periods of feeding (Table I). The lesser degree of filling of the stomach in the mornings is clearly shown by the smaller number of individual organisms consumed,

TABLE I. Numbers of food organisms of yellow perch are averages for 10 fish taken in two 48-hr periods, June 1–2 and 15–16, 1966. Per cent volume refers to entire sample.

Food item	6–9 PM (48 fish)		8–10 AM (31)	
	No.	% vol	No.	% vol
Cladocera	15	7	76	16
Ostracoda	3	< 1	1	
Amphipoda	10	8	3	10
Decapoda	1	3		
Isopoda	188	30	17	35
Ephemeroptera nymphs	2	5	3	20
Zygoptera nymphs	10	28	9	10
Anisoptera nymphs	1	10		
Chironomid larvae	3	4	13	4
Chironomid pupae	7	4		
Diptera adults			3	5

225 per 10 stomachs during the afternoon period and only 49 in the morning period if Cladocera be excluded. It is emphasized also by 188 isopods making up 30% of the total volume during the former and 17 individuals accounting for 35% during the latter.

Rock bass.— The major items of diet (Anisoptera nymphs, Decapoda, and fish) were the same at the three different times of day (Table II). Crayfish are most active at night. The large size of the items in the diet of this species is reflected in the small number of individual organisms consumed.

Pumpkinseed.— Here there was a clearcut shift in the major food items with hour of day (Table III). Predominant food items were: afternoon (3–5 PM), chironomid larvae, pelecypods, and Anisoptera nymphs; night (4 AM),

TABLE II. Numbers of food organisms of rock bass are averages for 10 fish taken in two 48-hr periods, June 1–2 and 15–16, 1966. Per cent volume refers to entire sample.

Food item	9–11 PM (43 fish)		1–4 AM (28)		7–10 AM (25)	
	No.	% vol	No.	% vol	No.	% vol.
Amphipoda			6	3		
Decapoda	2	10	7	30	5	50
Zygoptera nymphs			1	3		
Anisoptera nymphs	8	70	10	50	9	35
Trichoptera larvae	1		5	4	10	2
Chironomid larvae	1		5	3	5	2
Chironomid pupae	6		6	2	5	2
Ceratopogonid larvae					5	2
Neuroptera larvae					1	2
Fish	2	20	1	5	1	5

chironomid pupae, amphipods, and Trichoptera larvae; morning (5–8 AM), chironomid larvae, pelecypods, Zygoptera nymphs, Trichoptera larvae, and isopods. These shifts conform, in part, with what is known of cycles of activity in these food organisms. Thus chironomid and Trichoptera pupae pass vertically through the water column prior to emerging as adults mostly during the night, although smaller numbers do so in the forenoon and afternoon

TABLE III. Numbers of food organisms of pumpkinseed are averages for 10 fish taken in two 48-hr periods, June 1–2 and 15–16, 1966. Per cent volume refers to entire sample.

Food item	3–5 PM (46 fish)		4 AM (24)		5–8 AM (33)	
	No.	% vol.	No.	% vol.	No.	% vol.
Cladocera	5		42	2	1	
Copepoda			6	7	1	
Ostracoda			1		2	2
Amphipoda	7	4	10	17		
Decapoda			1	7		
Isopoda	1		2		6	10
Ephemeroptera nymphs	3		2	4		
Zygoptera nymphs			8	8	8	22
Anisoptera nymphs	6	34	1			
Trichoptera larvae	2		32	14	9	22
Chironomid larvae	190	35	7	6	25	27
Chironomid pupae	2	3	64	24		
Ceratopogonid larvae	1	3	2	4		
Gastropoda	4	2	7	5		
Pelecypoda	35	15	4		10	15
Hydracarina					2	2

(Ide, 1942). Amphipods and larval trichopterans may move up into the water column at night, the latter abandoning their cases to do so (Waters, 1962). The pumpkinseed, since it is a visual feeder, is probably best able to obtain bottom-dwelling organisms (chironomid larvae, pelecypods) when the light is good. A significant proportion of the organisms obtained at night, however, were apparently from the water column.

Bluegill.— In shallow water the predominant food items were: afternoon (3–5:30 PM), Cladocera, Anisoptera nymphs, and ceratopogonid larvae; middle of night (2–3 AM), ceratopogonid larvae; morning (5–9 AM), Cladocera, Ephemeroptera nymphs, ceratopogonid larvae, and chironomid pupae (Table IV). Thus, planktonic Crustacea were obtained during the daylight hours

TABLE IV. Numbers of food organisms of bluegill are averages for 10 fish taken in two 48-hr periods, June 1–2 and 15–16, 1966. Per cent volume refers to entire sample.

	Netted at 2–5 ft (seine) Birch Bay						Netted at 6–15 ft (gill) adjacent to Queen's University boathouse					
	3–5:30 PM (34 fish)		2–3 AM (23)		5–9 AM (38)		3–5:30 PM (21)		2–3 AM (22)		5–9 AM (41)	
Food item	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.
Cladocera	518	12	10		507	30	28	5	2		57	
Copepoda					3	2					1	
Ostracoda	3		1		1		13	2			8	10
Amphipoda	4		5	3	2	2	2				3	7
Isopoda					1		35	10	1		2	4
Ephemeroptera nymphs					114	25	2	2			12	15
Zygoptera nymphs			1	2	3	5	1	5			5	15
Anisoptera nymphs	8	35	1	2	1				2	15	1	2
Trichoptera larvae	18	3	4	3	7	4	19	10	36	55	8	20
Chironomid larvae	13		3	2	11	2	17	2	2		1	
Chironomid pupae	29	5	5	5	13	12	74	55	72	20	7	10
Chaoborus larvae					1						1	
Chaoborus pupae					1						2	
Ceratopogonid larvae	125	25	215	70	15	12			7	3	1	
Ceratopogonid pupae			12	3	1		7	4			10	6
Simulium larvae			1	2								
Diptera adults			1	2								
Hymenoptera adults	3	3	1				2				1	
Hemiptera			1		1							
Coleoptera	3	5									1	
Gastropoda					2	2			1	2	1	
Pelecypoda									3	5		
Hydracarina	4		1		7		4	2			2	
Araneida					1							
Fish eggs	187	10										

when they concentrated near the bottom, not from the water column at night, which is when the "water strainers" of Lake Opinicon, *Pomoxis nigromaculatus* and *Pomolobus pseudoharengus*, obtain them (Keast, 1968).

In the deeper water the major food items were: afternoon, chironomid pupae, Trichoptera larvae, and isopods; 2–3 AM, chironomid pupae, Trichoptera larvae, and Anisoptera; morning, Trichoptera larvae, Ephemeroptera nymphs, Zygoptera nymphs, chironomid pupae, and Ostracoda. Here the greatest difference lies in the more diverse range of organisms consumed during the daylight hours.

Relation of kinds of food to feeding time.— There can be no doubt that the different feeding times of the various species of Lake Opinicon fishes in a measure determine the kinds of food obtained, certain organisms being accessible, or most accessible, only at certain hours. Differences in feeding period ensure that all potential food organisms are cropped by one fish species or another. At the same time, at least in the case of the more versatile and generalized feeders, different feeding times must also serve to reduce interspecific contact and competition.

The daily shifts in diet demonstrated for the sunfish emphasize the shortcomings of basing food studies on fish collected at only one time of day. The danger is minimal in species that are either preponderantly diurnal or nocturnal and in specialized feeders. Even in the case of the former, however, care has to be exercised — note findings of Ide (1942) that trout (*Salvelinus fontinalis*) in Algonquin Park contained no *Simulium* in the morning, prior to their emergence, but large numbers at the height of the emergence during the forenoon.

ACKNOWLEDGMENTS

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Angler Harvest and Mortality of *Esox masquinongy* in Pigeon and Sturgeon Lakes, Ontario[1,2]

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ABSTRACT

In 1961 a creel census was begun on Pigeon Lake in south-central Ontario to assess the contribution to the fishery of maskinonge transplanted from nearby Nogies Creek Fish Sanctuary. A correction being made for a tag loss of 58%, it was estimated that 30% of the transplanted fish had appeared in the anglers' catch. The mean catch of maskinonge from Pigeon Lake for the seasons of 1961–65 was estimated to be 1318 fish per year. About 4% of the annual catch from Pigeon Lake was attributed directly to fish transplanted from Nogies Creek.

From a catch curve the rate of total mortality of Pigeon Lake maskinonge age V or older was estimated to be 43% per year. Partitioning this into mortality due to fishing and mortality from other causes yielded estimates of 24.5% for each of these components.

INTRODUCTION

During the last 20 years the Kawartha Lakes region of south-central Ontario has been the subject of an increasingly intensive sport fishery. One of the main attractions of this region is the occurrence of substantial numbers of maskinonge (*Esox masquinongy* Mitchill) in the lake system. In view of the popularity of the maskinonge as a game fish, it was thought that the population in the Nogies Creek Fish Sanctuary, a tributary of the lake system, might be able to contribute to the sport fishery.

Beginning in the fall of 1952 the Ontario Department of Lands and Forests tagged and transplanted juvenile and adult maskinonge from the Nogies Creek Sanctuary to the public fishing waters of Pigeon and Sturgeon lakes. The transplanting program has continued every fall since 1952 except 1956 and 1959 when maskinonge were trapped, tagged, and returned to the sanctuary for further study.

From tags returned to the Department of Lands and Forests, Muir and Sweet (1964) reported on the survival, growth, and movement of transplanted maskinonge in Pigeon and Sturgeon lakes. The tag returns provided a minimal estimate of the number of transplanted fish recaptured, but did not provide data for an estimate of the rate of tag loss. In 1961 a creel census was begun (Dursley and Fry, MS, 1961) on Pigeon Lake so that maskinonge taken in the fishery could be examined for identifying marks by a trained census clerk.

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The present paper utilizes creel census data from the summers of 1961–65 to evaluate the transplanting program in terms of angler harvest of Nogies Creek fish. The analysis includes an estimate of tag loss among transplanted fish and an estimate of the total catch of maskinonge from Pigeon Lake. Mortality rates of Pigeon Lake maskinonge were also determined and the results are compared with the corresponding parameters available for Nogies Creek fish.

MATERIALS AND METHODS

Pigeon and Sturgeon lakes (Fig. 1) are links in a chain of lakes and rivers forming the Trent–Severn Waterway in south-central Ontario. Sturgeon Lake is about 17 miles² in area and lies at an elevation of 812 ft. Pigeon Lake

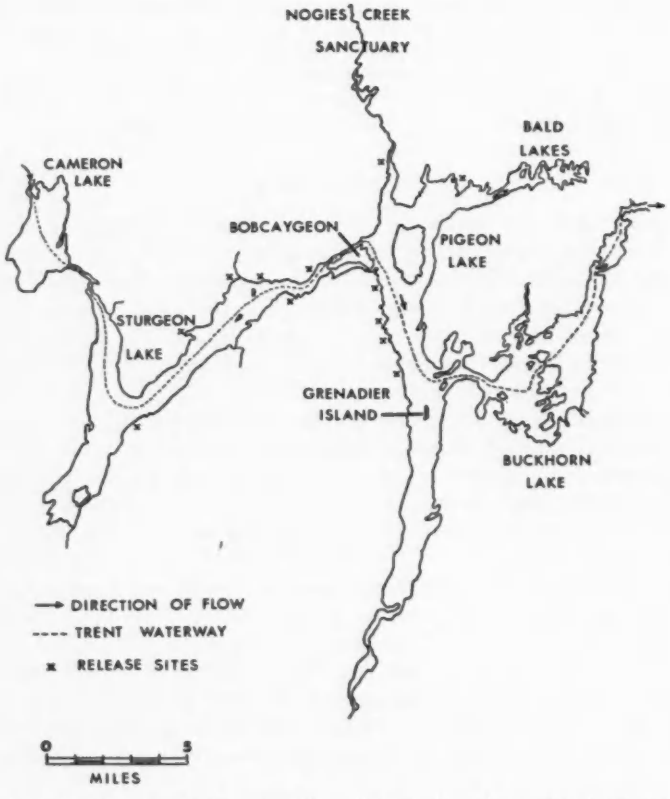

FIG. 1. Pigeon and Sturgeon lakes showing the release sites for transplanted fish. The dotted line indicates the course of the Trent–Severn Waterway. Arrows indicate the general direction of water flow.

is about 21 miles² in area and lies at an elevation of 805 ft. The lakes are separated by locks and a control dam on the Otonabee River at the Village of Bobcaygeon. Muir and Sweet (1964) estimated the flow of water at Bobcaygeon to be 1200–1500 ft³ per second during July and August. Both lakes are relatively eutrophic, supporting large areas of submergent aquatic vegetation. Pigeon Lake is continuous with the Bald Lakes and Buckhorn Lake.

The Nogies Creek Sanctuary has been previously described by Crossman (1956) and Muir (1963). The sanctuary consists of 4 miles of meandering stream terminating in an 80-acre lake. The stream is 20–30 yards wide with a mean depth of 10 ft. The average depth of the lake is about 8 ft. The maskinonge population is confined to the sanctuary by a rubble barrier at the upper end of the stream and a water level control dam at the outlet of the lake. Nogies Creek from the lower boundary of the sanctuary to its outlet into Pigeon Lake is open to angling during the regular fishing season.

Transplanting Procedure

The sanctuary population was harvested each fall with trap nets set by the Department of Lands and Forests. The nets were usually set in October and fish were either transplanted on the day of capture or held in retainer nets for transplanting the following day. The policy of the Department of Lands and Forests was to transplant maskinonge 20 inches or longer (total length to tip of caudal fin) and to return smaller fish to the sanctuary. The minimum legal length for maskinonge in the Kawartha Lakes region is 28 inches. Fish to be transplanted were finclipped and then tagged with a numbered plastic disc. The distal half of the fin to be clipped was removed with sharp scissors so that a clean cut was made. Finclips in successive years were rotated clockwise (from dorsal aspect) through the paired fins. Prior to 1963 the tag was affixed to the preopercle bone with a loop of monofilament nylon (Crossman, 1956), but in 1963 and subsequent years the tag was attached immediately anterior to the dorsal fin with the monofilament loop passing behind the anterior pterygiophore. Length and weight data and a scale sample for age determination were taken at the time of tagging.

A total of 3474 tagged maskinonge were transplanted from the sanctuary to public fishing waters from 1952 to 1964 (Table I). Of these fish 53 were released in Cameron Lake and the remaining 3421 fish were nearly equally distributed between Pigeon and Sturgeon lakes. No attempt was made to allocate fish of a certain size to either of the lakes.

Tag Recovery

Some of the transplanted fish presumably lost their tags prior to the next fishing season. In sanctuary fish Muir (1963) observed that a scar was produced by the monofilament loop even if the tag was lost within a few weeks after tagging. Since tag scars would not be obvious to the casual angler, the creel census was designed to allow inspection of a maximum number of maskinonge by a trained observer. Finclips were particularly useful in identifying

TABLE I. Numbers of tagged maskinonge transplanted to Pigeon and Sturgeon lakes from 1952 to 1964 and subsequent tag returns by year of recapture. Data for the years 1952–58 are from Muir and Sweet (1964).

Year of plant	No. planted	% return	Year of return												
			1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965
1952	155	11	16	0	1										
1953	133	23		26	4	1									
1954	131	15			15	3	1	1							
1955	298	18				41	10	1	1						
1956	0														
1957	323	13					1	39	3						
1958	493	13						1	59	3					
1959	0														
1960	376	11									35	5			
1961	320	11										27	9		
1962	512	9											35	10	
1963	378	18											3	43	22
1964	355[a]	8[b]													27
Total	3474	13	16	26	20	45	12	42	63	3	35	32	47	53	49

[a]Of these fish, 53 were transplanted to Cameron Lake (Fig. 1).
[b]Returns from 1965 only.

fish which had borne tags in the dorsal position since these tag scars were very obscure. The census clerk took head length, fork length, and total length measurements and collected a scale sample from each fish encountered. During the 1965 census, weight data were also collected.

The most productive part of the creel census was a boat patrol on Pigeon Lake north of Grenadier Island (Fig. 1). An outboard runabout allowed the census clerk to contact anglers while they were fishing and provided transportation to tourist resorts and fishing camps. The lake was patrolled daily, weather permitting, from the opening day of the maskinonge season (usually the last Saturday in June) until about the second week in September. In addition to information obtained through the boat patrol, many fish were made available for inspection through the cooperation of fishing camp operators and guide-boat operators who informed the census clerk of their parties' catches.

The study was publicized locally by the Bobcaygeon Chamber of Commerce and by posters announcing the annual tag draw contest sponsored by the Toronto Anglers' and Hunters' Association. A study of game fish in the Kawartha Lakes by the Department of Lands and Forests ran concurrently with the maskinonge project and publicized the importance of reporting tags.

Age Determination

Scale samples collected during the creel census and transplanting procedure were prepared for age determination by making impressions of the scales in soft acetate plastic slides after the procedure described by Arnold (1951). The slides were read by means of a Leitz "Trichinoskop" projector. Muir's scale collection from the 1959 fall trapping in the sanctuary was reread to provide a basis of comparison for ages assigned by Muir and those assigned in the present study. The scale samples from 17 of the transplanted fish were regenerated or otherwise unsuitable for age determination.

RESULTS

Contribution of Transplants to the Fishery

Excepting the 1964 transplant, for which returns were available for only 1 year after transplanting, the tag returns from Pigeon and Sturgeon lakes varied between 9 and 23% of the number of fish transplanted and averaged 13% (Table I). Most of the tag returns (363 out of 443, or 82%) were from the year after the transplant, but tagged fish were recaptured as late as four seasons after transplanting.

Large plantings of age II fish were made in 1958 and 1962, but most of the fish were transplanted at ages III, IV, and V (Table II). The data for fish age X and older are too few to be meaningful, but for age-groups II–IX there was an apparent increase in the percentage of tags returned from fish of successively older age-groups at the time of transplanting (Fig. 2).

TABLE II. Numbers of tagged maskinonge of each age-group transplanted (T) to Pigeon and Sturgeon lakes and subsequent recaptures (R). Data for the years 1952–58 are from Muir and Sweet (1964).

Year of plant	Data	Age at transplanting													
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIV	Total
1952	T	0	3	17	60	39	27	9							155
	R			2	2	5	8								17
1953	T	0	1	13	44	56	18	1							133
	R			1	10	17	3								31
1954	T	0	2	16	49	32	24	4	2	0	1	1			131
	R				9	6	4	1							20
1955	T	0	5	43	82	94	48	16	5	1	1	2	1		298
	R			2	6	20	15	8	2						53
1957	T	0	0[a]	1	45	109	77	58	27	2	3	0	0	1	323
	R					10	13	12	7					1	43
1958	T	0	156	150	89	57	25[a]	9	4	3					493
	R		9	11	12	19	5	4	1	2					63
1960	T	0	8	112	149	65	21	7	3	2					367
	R		0	5	16	7	9	2	0	1					40
1961	T	0	48	73	123	62	10	4							320
	R		1	6	13	13	2	1							36
1962	T	4	163	128	86	74	40	8	4	2					509
	R		5	6	13	6	9	4	1	1					45
1963	T	3	36	118	96	66	31	16	6	2	1				375
	R		4	20	14	18	10	1	1						68
1964	T[b]	4	91	74	85	43	31	16	8	0	0	0	1		353
	R		4	2	7	7	3	3					1		27
Planted:		11	513	745	908	697	352	148	59	12	6	3	2	1	3457
Recaptured:		0	23	55	102	128	81	36	12	4	0	0	1	1	443
Percentage:		0	4.5	7.4	11.2	18.4	23.0	24.3	20.3	33.3	0	0	50.0	100.0	12.8

[a]The value published here in Muir and Sweet (1964) was the result of an error in transcription (B. S. Muir, personal communication)
[b]Of these fish, 53 were transplanted to Cameron Lake (Fig. 1).

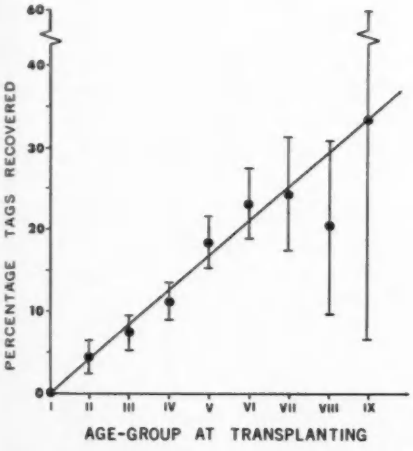

FIG. 2. Percentage of tags recovered for each age-group transplanted. The 95% confidence intervals are indicated by I-bars.

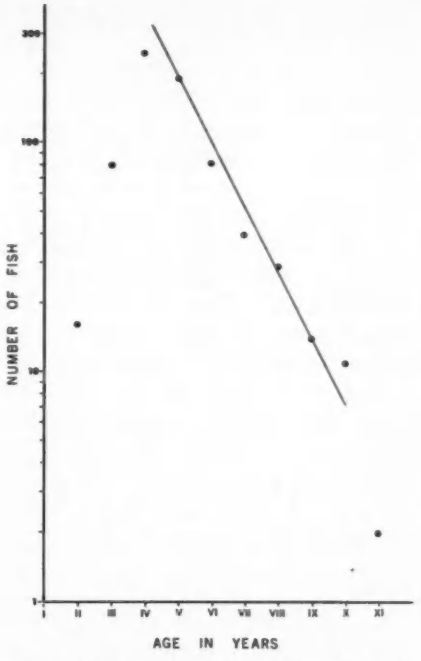

FIG. 3. Catch curve for the Pigeon Lake maskinonge for the years 1961–65. The line was fitted by the method of least squares for age-groups V–X.

To estimate the contribution of each age-group to the fishery it was necessary to correct for tag loss among the transplanted fish. Of the 40 fish in the creel census known to have been tagged, only 17 or 42% still bore their tags when examined by the census clerk (Table III). This means that if the rate of tag loss was constant for all ages, the percentage of each age-group actually recaptured was approximately twice that indicated by Fig. 2. Doubling the percentage return values in Fig. 2 indicates that transplanting maskinonge younger than age V yielded returns to the angler of less than 20%; for age V fish, at least 35%; for age-groups V and older, almost 50%.

The total number of transplanted maskinonge captured in the fishery was estimated by applying the tag loss estimate to the number of tags returned. If the 443 tags returned to the Department of Lands and Forests (Table II) represented 42% of the transplanted fish recaptured, the catch of transplanted maskinonge was 1055, or 30% of the fish transplanted from 1952 to 1964.

CATCH ESTIMATE FOR PIGEON LAKE

If the tags returned to the Department of Lands and Forests from fish caught in Pigeon Lake from 1961 to 1965 represented the same proportion

of these fish, 53 were transplanted to Cameron Lake (Fig. 1).

TABLE III. Summary of marked and unmarked maskinonge observed in the Pigeon Lake creel census from 1961 to 1965 and the total tag returns by year from maskinonge caught in Pigeon Lake. The "unmarked" fish bore no tags, tag scars, or finclips whereas the "marked" fish bore finclips or tag scars, but not tags.

Census season	No. of maskinonge observed				Tags returned
	Unmarked	Tagged	Marked	Total	
1961	119	5	4	128	24
1962	129	6	9	144	24
1963	114	2	2	118	36
1964	189	1	3	193	31
1965	227	3	5	235	22
Total	778	17	23	818	137

of the total catch as tagged fish did of the creel census (Table III), then the total catch of maskinonge from Pigeon Lake for these years, estimated by simple proportion, was $137 \times 818 \div 17$, or 6592. The estimated average catch per year was then 1318 with a 95% confidence interval of 895–2505. Since no tagged maskinonge were reported from Pigeon Lake south of Grenadier Island, the catch estimate applies only to the area covered by the boat patrol. This estimate relies heavily on the assumption that all tagged fish caught were reported to the Department of Lands and Forests. Since this condition is seldom met in a fishery having multiple access points, the estimate obtained is taken to be minimal.

MORTALITY ESTIMATES

The rate of total mortality of Pigeon Lake maskinonge was estimated by constructing a catch curve (Fig. 3) based on the age composition of the unmarked fish observed in the creel census. Data for the years 1961–65 were combined in order to minimize the effects of fluctuations in year-class abundance. The slope of the line represents a total annual mortality rate of 43% for age-groups V–X.

The total annual mortality rate was divided into a fraction due to fishing and a fraction due to natural causes by applying the equation for competitive mortality. The relationship is: $a = m + n - mn$, where a is the total annual mortality rate; m is the rate of mortality due to fishing; and n is the rate of mortality due to natural causes (Ricker, 1958). If 50% of the transplanted fish age V or older were recaptured by anglers, then m equals n. If a equals 0.43, then the equation becomes: $n^2 - 2n + 0.43 = 0$. Solving for n, the annual mortality rate due to natural causes is 24.5%.

DISCUSSION

Heacox (1946) reported an average catch of 1745 maskinonge per year for Chautauqua Lake, New York, over the period 1941–44. His description

of Chautauqua Lake, 21 miles² in area, relatively shallow, and with dense beds of aquatic vegetation closely resembles that of Pigeon Lake. The Chautauqua Lake catch figures were based on reports required of all fishermen licensed to fish for maskinonge in the waters of Chautauqua and Cattaraugus counties. The Chautauqua Lake catch figures were for maskinonge 32 inches or longer (total length) and the Pigeon Lake catch estimate was for fish 28 inches or longer.

The results above indicate that about a third of the fish transplanted under the current management program ultimately contribute to the sportsman's catch. If an average of 300 fish could be transplanted annually to Pigeon and Sturgeon lakes, then the annual contribution to the catch would be about 100 fish. Assuming that the recapture rates are equal in Pigeon and Sturgeon lakes (Muir and Sweet, 1964), the 50 fish contributed to the Pigeon Lake catch would represent about 4% of the annual harvest from Pigeon Lake.

The contribution to the fishery might be increased by transplanting more older fish from the sanctuary, but to do so could possibly limit reproduction in the sanctuary population. Fingerling maskinonge from the provincial hatchery at Deer Lake have been planted in the sanctuary annually in an attempt to see if Nogies Creek might serve as a nursery area for rearing maskinonge to a "transplanting size."

The total mortality rate of Pigeon Lake maskinonge age V and older does not seem to be exceptionally high. Although he placed no great confidence in his estimate of natural mortality, it is interesting to note that Muir (1964) obtained an annual natural mortality rate of 24% for Nogies Creek maskinonge age V and older. The natural mortality rates of maskinonge in Pigeon Lake and in the sanctuary are apparently of the same order of magnitude.

ACKNOWLEDGMENTS

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Relation of Pure Tone Thresholds to Background Noise Level in the Atlantic Cod (*Gadus morhua*)[1]

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ABSTRACT

A conditioning procedure was used to determine auditory thresholds of cod at frequencies 35.3, 70.7, 141, 283, and 400 Hz at four levels of background noise. The noise was produced in octave bands centered at the test frequencies, and ranged in level from −14 db to +4 db (re 1 microbar). Up to 283 Hz thresholds varied directly with background noise, and signal-to-noise levels at thresholds remained approximately the same at all background noise levels for each frequency. Thresholds at 400 Hz were much higher and showed no relationship to noise. Mean signal-to-noise levels are calculated.

INTRODUCTION

Numerous studies on the auditory capacities of fish have been carried out. Most of them, however, were carried out in tanks in which special efforts had been made to reduce background noise (Enger, 1966; Moore and Newman, 1956; Tavolga and Wodinsky, 1964, 1965; Wisby et al., 1964). These methods result in audiograms of the species concerned, but give little information on the ability of the fish to detect signals in their natural environment.

Recent experiments (Buerkle, 1967; Jacobs and Tavolga, 1967) point to the need to relate thresholds to background noise. The present study attempts to determine thresholds of cod (*Gadus morhua* L.) for pure tone signals in varying levels of background noise, and to relate this information to the ability of cod to perceive signals in their noisy natural environment, the oceans.

METHODS

The cod were captured in Passamaquoddy Bay, N.B., by otter trawl and kept in the laboratory as previously described (Buerkle, 1967). The experimental tank has also been described, but for this experiment it was better insulated from noise by setting it on thicker pads of vibration absorption material (Tico-mat, James Walker & Co. Ltd., Surrey, England), and by enclosing it in a plywood box lined with 2 inches of fibreglass.

Thresholds were determined by a conditioning procedure which employed electric shock to train cod to change their cardiac rhythm in response to sound stimuli (Buerkle, 1967).

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The aim in this study was to determine thresholds at frequencies 35.3, 70.7, 141, 283, and 400 Hz at varying levels of background noise. Due to the characteristics of the sound-producing system, a background noise field that was flat over the whole desired frequency range could not be produced in the experimental tank. Therefore, for each threshold determination the background noise was produced in the octave band centred so that the test frequency was located at its geometric midpoint.

The background noise-producing system consisted of a GR model 1390B random noise generator (Fig. 1). Its output was filtered to the desired octave band by an Allison model 2AB band pass filter, and was amplified by one channel of a stereo amplifier (with a flat response from 20 to 20,000 Hz).

The pure tone test signal was produced by a Hewlett–Packard model 205AG signal generator and amplified by the other channel of the stereo amplifier.

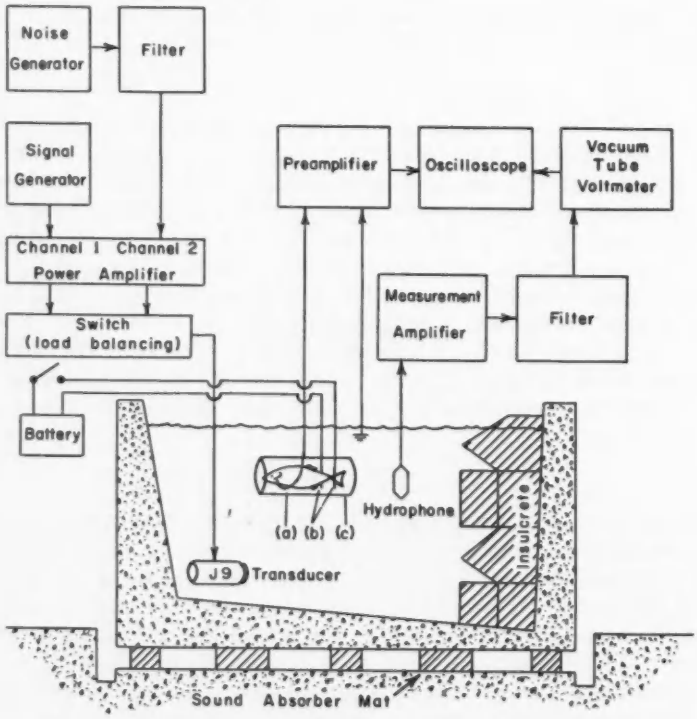

FIG. 1. Block diagram of instrumentation (see text for details), and cross-section of experimental tank. Electrode (*a*) is implanted in the pericardial region of the fish to obtain the electrocardiogram, electrodes (*b*) are attached to plastic screen cage (*c*) so that the unconditioned stimulus can be applied to the caudal peduncle of the fish.

To prevent a possible orientation response of the cod to test signals produced on a speaker other than that producing the background noise, the same speaker (Chesapeake Inst. Corp. Model J9) was used for both test signal and background noise. Since the test signal had to be turned on and off while the background noise had to be produced continuously at a steady level, a load balancing and switching transient eliminating circuit was used between the outputs of the stereo amplifier and the underwater speaker (Fig. 1).

The various gain controls of the system were set so that pure tones at the test frequencies (35.3, 70.7, 141, 283, and 400 Hz) could be produced at pressure intervals of 2 db, and background noise in bands 25–50, 50–100, 100–200, 200–400, and 283–566 Hz could be produced at pressure levels of −14, −8, −2, and +4 db (re 1 microbar).

For each fish a threshold was determined at each of the test frequencies in ascending order. Each fish was tested at one background noise level only. For each test, the background noise was turned on 30 min (arbitrarily chosen) before testing was begun, and kept on to the conclusion of that test. The process was continued until three different fish had been tested at each level of background noise.

RESULTS

For frequencies 35.3–141 Hz average thresholds were higher at the higher noise levels, but seemed constant for each noise level (Fig. 2). The variation of thresholds at the −8 db noise level is considered (by analysis of variance) not to be significant at the 5% level. At 283 Hz the average threshold at the −14 db noise level was 6 db above that at the −8 db noise level. In view of the small sample size (three) making up each point, appreciable variation is to be expected. Since, of the four levels of threshold at 283 Hz, only one is out of order, the rule of higher threshold at higher noise levels is expected to apply to 283 Hz as well as the frequencies below.

At 400 Hz, however, there is no obvious relationship between threshold and background noise level. This is consistent with previous work (Buerkle, 1967) in which thresholds of cod at 400 Hz were found to be uninfluenced by background noise. From the present experiment it can be concluded therefore that the absolute threshold of cod at 400 Hz with its 95% fiducial limits is 23.0 ± 2.3 db (re 1 microbar).

The average difference between thresholds at adjacent noise levels for frequencies 35.3–141 Hz is 5.3 db. This differs little from the 6 db which separate adjacent noise levels, and indicates that threshold varies directly with background noise. The signal-to-noise level was calculated for each threshold measurement (Table I). At frequencies 35.3–283 Hz there was no significant difference at the 5% level (analysis of variance) between signal-to-noise levels for different background noise levels within each frequency. In this frequency range, signal-to-noise levels of cod thresholds therefore seem constant for thresholds determined in background noise at levels from −14 db to +4 db.

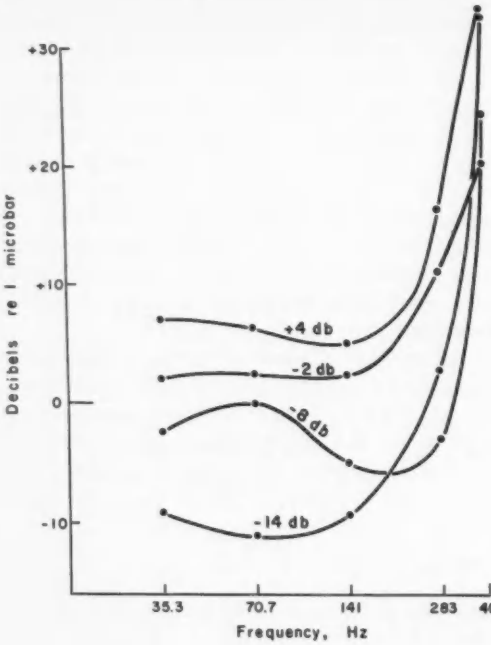

FIG. 2. Mean thresholds of cod at four levels of background noise.

TABLE I. Average signal-to-noise levels for thresholds at four levels of background noise.

Noise level (*db re 1 microbar*)		Signal to noise in octave bands (*db*)				Signal to noise in 1-Hz bands (*db*)			
		−14	−8	−2	+4	−14	−8	−2	+4
Frequency	35.3	5.0	5.7	4.0	3.0	19.0	19.7	18.0	17.0
	70.7	5.0	8.0	4.3	2.3	20.0	25.0	21.3	19.3
	141	4.7	3.0	4.3	1.0	24.7	23.0	24.3	21.0
	283	17.0	5.0	13.3	14.7	40.0	28.0	36.3	37.7
	400	37.0	25.7	22.5	29.5	61.5	50.2	47.0	54.0

To correlate the results of this experiment with noise levels in the oceans, which are usually described in spectrum levels (i.e., 1-Hz bands), the noise levels of this experiment (measured in octave bands) were converted to 1-Hz levels. Signal-to-noise levels were calculated for these 1-Hz band noise levels (Table I). Average signal-to-1-Hz-noise levels and their 95% fiducial limits were calculated for each frequency (Fig. 3). The average ratio for frequencies

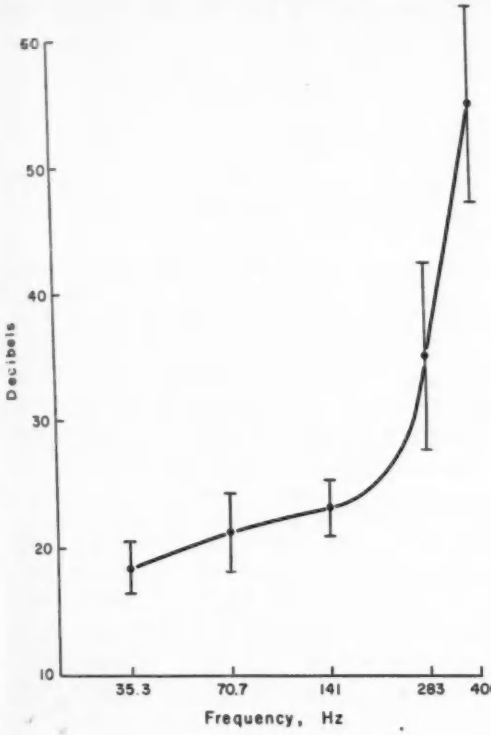

FIG. 3. Mean signal to 1-Hz noise levels of thresholds and their 95% fiducial limits.

35.3–283 Hz in this experiment was 24.9 ± 2.7 db which falls well within the 28.7 ± 6.6 db limits for frequencies 17.6–283 Hz calculated in previous work (Buerkle, 1967).

DISCUSSION

Noises in the oceans are highly variable (Wenz, 1964). In the frequency range between 35 and 400 Hz, the upper limit of prevailing noise spectrum level decreases from about −4 db (re 1 microbar) at 35 Hz to about −19 db (re 1 microbar) at 400 Hz. The sources producing prevailing noise in this frequency range are: turbulent pressure fluctuations, oceanic traffic, surface agitation, and seismic background. Those contributing intermittently are: earthquakes, biological sources, precipitation, and ship and industrial activity (Wenz, 1964). At any one time, at any one place, any or all of these sources may be operative.

Since the auditory thresholds of cod at frequencies up to 283 Hz vary with background noise level at least between −14 and +4 db (octave level), and the upper limit of this noise range corresponds to a spectrum level of

−10, −13, −16, and −19 db in noise bands 25–50, 50–100, 100–200, and 200–400 Hz respectively, and these levels fall below the upper limit of prevailing noise, statements concerning the auditory capacity of cod should be related to background noise. A knowledge of the ratio between threshold and background noise spectrum level enables one to make meaningful statements concerning the auditory capacity of fish in their natural environment.

The present experiment has attempted to define such a relationship, and the results are expected to aid in answering such questions as "can cod detect noises produced by fishing gear?"

The relationship between threshold and background noise must change, however, when the absolute threshold is reached. At this point further decreases in background noise will not result in decreases of threshold. The signal-to-noise levels, therefore, will increase as background noise level is lowered. To determine the lower limit to which the signal-to-noise levels calculated in this study are likely to apply, a knowledge of the absolute threshold is necessary. The determination of absolute threshold would entail decreasing background noise levels until signal-to-noise levels showed corresponding increases. However, making experimental tanks quiet enough, and producing test signals at these low levels is extremely difficult and was impossible with available equipment.

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Distribution of the Parasitic Isopod *Hemioniscus balani* with Special Reference to the East Coast of North America[1]

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ABSTRACT

Hemioniscus balani Spence Bate, a parasitic isopod with a world-wide distribution in temperate waters, was not found among intertidal populations of *Balanus balanoides* examined between 1959 and 1965 on the east coast of North America from Newfoundland to Cape Hatteras, except for a small area near Halifax, Nova Scotia.

The North American variety of *Balanus balanoides*, when transplanted from Woods Hole to North Wales, was found to be much more susceptible than the European variety to parasitisation by *H. balani*.

INTRODUCTION

Hemioniscus balani is an isopod parasite living in the mantle space of many species of sessile barnacles. It is widespread in Europe and the same or similar species has been observed in South Africa (Sandison, 1954) and the Pacific coast of North America in *Chthamalus dalli* (Cornwall, 1955). It was not recorded by Bousfield (1954) who examined the reproductive condition of several hundred specimens of five species of barnacles, four of which were potential hosts.

The adult undergoes sex change from a fusiform male to a featureless, bloated female. The latter can readily be seen as a yellow or orange mass filling or partly filling the mantle space when the host barnacle is removed from its substratum and examined by the naked eye. Sample estimates of the percentage infection by females in a barnacles population can therefore readily be made in the field. All the data given in this paper refer to the number of mature-size barnacles infected by the female cryptoniscid parasite; the true rate of infection by both sexes would presumably be higher.

Observations on the North America Coast

In the course of several journeys over the period 1959–63 along the east coast of North America between Newfoundland and Cape Hatteras, the southern limit of *Balanus balanoides* (Wells et al., 1960), counts of *Hemioniscus* in populations of *B. balanoides* were made at many locations. The observations are included in the data shown in Fig. 1 and 2. As can be seen, the species appears to be almost universally absent apart from a small enclave centered near Halifax in Nova Scotia. *Balanus balanoides* samples collected from several

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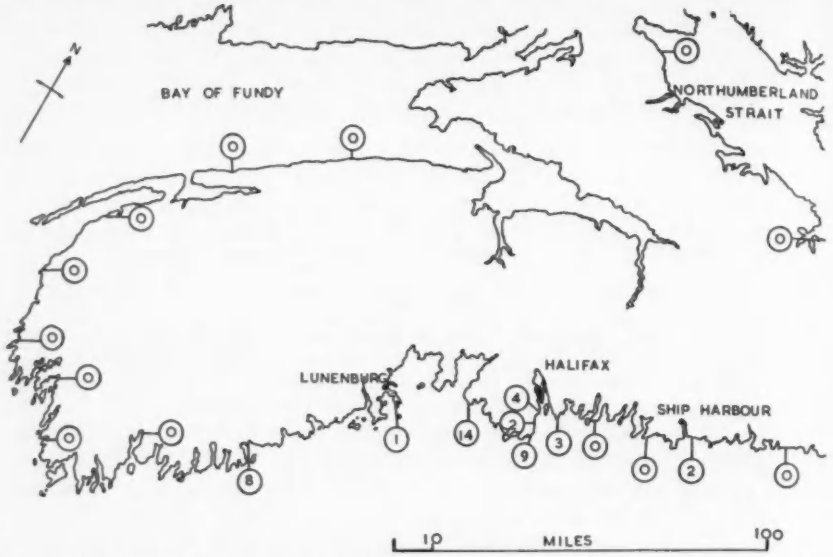

FIG. 1. Distribution of *Hemioniscus* in Nova Scotia giving the percentage infection at various localities. All samples examined comprised at least 100 adult *Balanus balanoides*. No *Hemioniscus* was found to the east of the area shown.

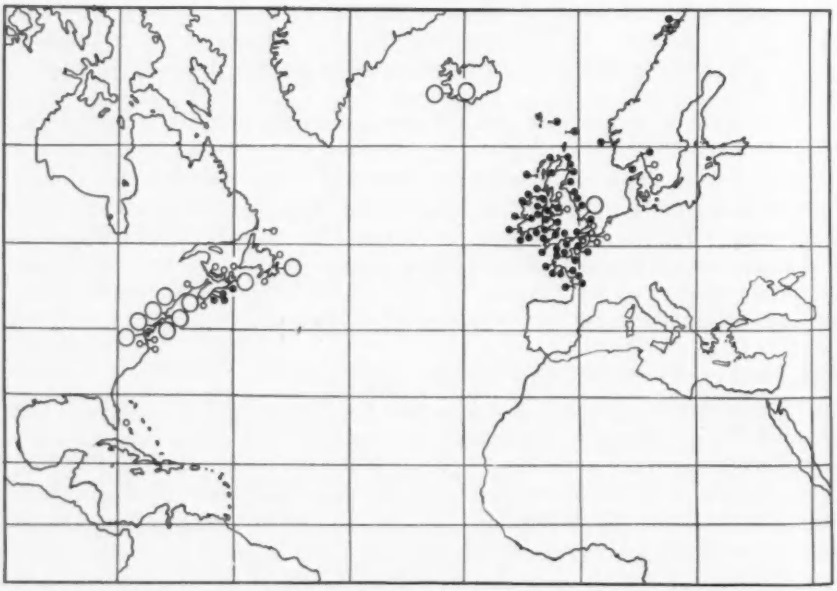

FIG. 2. Distribution of *Hemioniscus balani* in the North Atlantic. Solid circles: records of *Hemioniscus*; small open circles: no *Hemioniscus* in samples of at least 100 barnacles; large open circles: no *Hemioniscus* in samples of at least 500 barnacles.

sites near Reykjavik, Iceland, have also been examined for *Hemioniscus*. Of 900 specimens from six localities none contained parasites. The observations in Newfoundland, Iceland, and the eastern coast of the United States were made in the summer months (July, August); those in Maine and Nova Scotia in the autumn (September–November).

Observations on Transplants

A number of pieces of rock and shells covered with *B. balanoides* were transported from Cape Cod, Massachusetts, to the Menai Straits, North Wales, in the summers of 1959 and 1960. A large number of individuals were sampled before transplantation and were found to be without infection by *H. balani*. Intensive field observations in which well over a thousand individuals were examined also failed to reveal the parasite in the Cape Cod area.

After transplantation, samples both of transplanted and native barnacles were removed at intervals and the rate of parasitisation measured. The results are given in Table I. At the time of transplantation the barnacles were free from infection, but within about 6 months a level of infection of about 5%

Table I. Infection of *Balanus balanoides* by *Hemioniscus balani* after transplantation from Woods Hole to Menai Bridge.

Date transplanted from America	Date examined in North Wales	No. and % infected in Transplanted specimens		No. and % infected in Native specimens	
			%		%
August 1959	August 1959	0/200	0	2/350	0.6
" "	December 1959	4/82	5	0/259	0
" "	December 1960	13/56	23	0/166	0
June 1960	December 1960	9/124	7	1/252	0.4

had been reached. Though this level is not high, it was many times greater than that of barnacles indigenous to the Menai Straits and living adjacent to the transplants. A year after transplantation, when the North American individuals had reached a large size, at least a fifth were infected, whereas the native barnacles adjacent to them were still almost free of infection.

In order to eliminate, as far as possible, the possibility that some of the barnacles were carrying the infestation on arrival in Britain, a further survey in the Woods Hole area was undertaken in the summer of 1965. Over a thousand individuals were examined from the two localities in which the original consignments had been collected, both in the field for female *Hemioniscus*, and in the laboratory for males and females, but none was found. A smaller sample of 150 collected in Woods Hole in November 1961 was also found to be free of infection.

Distribution of *Hemioniscus balani*

The obvious conclusion to be drawn from the above observations is that *H. balani* is extremely rare or absent from the barnacle *B. balanoides* over the whole of the eastern coast of North America, except for a relatively small area along the west central coast of Nova Scotia. Here the level of infestation, particularly on exposed rocks at Peggy's Cove, was high.

One reservation must be made: that since the great majority of observations in which *Hemioniscus* was found to be absent were made in the summer months, whereas those in which *Hemioniscus* was present were made in October, there is the faint possibility that the parasite occurs seasonally. However, in Europe this parasite is to be found in most localities at all times of the year and is common in summer as far south as the river Gironde, where the normal host, *B. balanoides*, terminates (Crisp and Fischer-Piette, 1959). I have also recorded it in summer at least as far north as Oslo Fjord. Furthermore, many barnacles were examined in other parts of Nova Scotia during October 1961, without finding the parasite, nor was it found in November and December in several hundred barnacles collected in Conception Bay, Newfoundland. Hence the limitation in the number of sightings of the parasite to one area seems likely to reflect a genuine restriction in its distribution and not a seasonal effect.

Hemioniscus balani infects only certain species and is never found, save accidentally, among others (Perez, 1923; Crisp and Fischer-Piette, 1959). Many of the species readily infected are sublittoral, including *Balanus balanus* which is common along the North American coast from Labrador to Massachusetts. This species has not been intensively examined for the parasite west of the Faroes, where I have found up to 8% of the individuals collected at depths of 100–150 m to be infected. Had this very obvious parasite been present in any abundance, however, it is surprising that it has escaped being noticed and recorded.

The most northerly point at which I have recorded *Hemioniscus* in Europe was in *B. balanoides* from Tromsø (69°N lat) in a series of collections made for me by Soot-Ryen in 1951; it was present there in less than 1% of the sample, and even rarer in samples from Espegrend, near Bergen. I have not found the parasite in the Baltic nor in the Kattegat, but it is not uncommon at the entrance to the Skaggerak and in the Oslo Fjord. It appears to be rare or absent from the Dutch and German coasts. It is common throughout Britain, except in the southeast, and is very common in the southwest (Crisp and Southward, 1954) and on the Atlantic coast of France (Crisp and Fischer-Piette, 1959). Nothing is known of its latitudinal distribution in the Pacific, except that it is very common in *Ch. dalli* and occasionally present in *Balanus glandula* at low tidal levels at Friday Harbour, Washington. I have recorded it commonly in the sublittoral species *B. balanus* and *Balanus hameri* in the Irish Sea, 54°N, and also in the former species in the vicinity of the Faroe Islands (62°N, 6°W) and Shetlands (61°N, 1°W). It was not found in collections made southwest of Bear Island nor near Spitzbergen, nor intertidally in Iceland. I have never found it in barnacles growing in the tropics nor in the subtropics.

It is noteworthy that it has not been recorded from *Balanus amphitrite* Darwin (= *B. amphitrite* var. *denticulata* Broch) living on the east coast of the United States. This species can readily be attacked in areas where the parasite is present, as in warmed effluents in Britain (Crisp and Molesworth, 1951). Nor has the parasite been observed among many specimens of *Chthamalus fragilis* and *Chthamalus stellatus* var. *angustitergum* Darwin which the author has examined, though it is possible that these species, like *Chthamalus stellatus stellatus*, are immune from attack.

The above records as set out in Fig. 1 suggest that the parasite is limited to cool, temperate, and equable climates.

The absence of the parasite over such a large area of the eastern American coast with a local population near Halifax suggests either a recent introduction or some locally favourable condition allowing intertidal populations, recruited perhaps from the sublittoral, to survive. The Atlantic coast of Nova Scotia is, however, colder in winter though slightly warmer in summer (Bousfield, 1962; Fuglister, 1947) than the Bay of Funday, where *Hemioniscus* was not found. Hence there is no obvious reason why the Halifax area should be more suitable than the south of Nova Scotia (Cape Sable), the Bay of Fundy, or Cape Cod, where it is also apparently absent. If warmer summers favoured the parasite by increasing its reproductive rate, areas to the south of Nova Scotia would then offer a more advantageous environment. Similarly, if the absence of the parasite from most of the east coast of North America was a result of its wider seasonal extremes of temperature compared with those of European and Pacific coasts, it seems unlikely that the Halifax area alone would provide just the range of extremes that it could tolerate.

More probably, therefore, the occurrence of the parasite in the area results from a recent introduction. Halifax is a busy port, since it remains ice-free in winter, and would offer the obvious place of entry of a barnacle parasite, though Lunenburg and other small ports are a possible primary or secondary centres of dispersal. The immediate vicinity of the harbour was not the area of highest infection but rather the exposed coasts outside were (Fig. 2); this is not an unusual feature in the distribution of *Hemioniscus* and may be related to milder winter conditions on exposed promontories (Crisp, 1964).

In the long term, Nova Scotia may prove unsuitable. Further observations are therefore needed to ascertain whether the parasite is now spreading, whether it forms a confined stable population, or whether it was a transient phenomenon. In the latter event, observations on sublittoral populations of *B. balanus* would be particularly revealing, as these might provide a reserve population of parasites from which infections of intertidal populations could arise from time to time.

Susceptibility to Infection

Table I shows clearly that the transplanted barnacles from Woods Hole became very much more heavily infected by *H. balani* than did the samples

of native barnacles collected simultaneously. The remote possibility that transplants had been already infected at Woods Hole is rendered even less likely by the fact that transplanted individuals examined over a year after arrival, displayed a much higher rate of infection than those examined 6 months after arrival (Table I, rows 2 and 3). On similar grounds it seems most unlikely that the process of transplantation itself rendered this group of barnacles unduly susceptible. Not only did they show little mortality, but they grew well and bred normally during the autumn after their arrival. Furthermore, many transplantation experiments in which a similar period of time was spent in transport have been carried out from one part of the British coast to another with no consequent increase in infection having been noted.

Field observations suggest that *Hemioniscus* is not indigenous to the east coast of North America. The variety of the host organism *B. balanoides* that is found on this coast differs genetically from the European variety (Crisp, 1964). It seems very likely, therefore, that the greater susceptibility to infection by the parasite of the American form compared with that of its European counterpart results from lack of exposure to the parasite. Though nothing is known of the means of resisting infection, such mechanisms must exist since some species are known to be susceptible whereas others are not. Susceptible species (*B. balanoides*, *B. glandula*, *B. balanus*, *B. hameri*, *B. amphitrite amphitrite*, *B. improvisus*, *B. algicola*, *Chthamalus dentatus*, *Ch. dalli*, *Elminius modestus*) do not differ in any obvious phylogentic, ecological, or morphological respect from those which are immune (*B. crenatus*, *B. perforatus*, *Ch. stellatus*, *Verruca stroemia*). Individuals that are infected are usually rendered sterile. In consequence, resistant varieties would be selected where the parasite was common, although genetic drift might allow susceptible varieties to appear in the absence of the parasite. It is suggested that this has happened on the eastern coast of North America.

ACKNOWLEDGMENTS

The investigation was made possible through many generous grants which gave me the opportunity to do field work along much of the Canadian and American east coast. Among these were grants from the Royal Society of London and the University of Wales; the Systematics-Ecology Programme of the Marine Biological Laboratory, Woods Hole, Mass., which supported travel in the United States; and the Nuffield Foundation which, jointly with Dalhousie University, Halifax, N.S., and Memorial University of Newfoundland, St. John's, Nfld., supported a tour of the maritime provinces of Canada.

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Sampling Larval Herring from Tidewaters with Buoyed and Anchored Nets[1]

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ABSTRACT

Buoyed and anchored nets were evaluated as a method of sampling larval Atlantic herring, *Clupea harengus harengus* Linnaeus, in tidewaters. The method permitted exploratory sampling in areas difficult to reach with towed gear, and provided a large number of samples from predetermined depths without towing from vessels. The nightly variation of catch rates was small under similar tidal conditions, and seasonal changes in larval catches were similar to those determined by towing nets from a boat. The buoyed net also provided information relating the distribution of larvae to tidal currents that could not be readily obtained with towed gear.

INTRODUCTION

To study the early life history of Atlantic herring, *Clupea harengus harengus* Linnaeus, in the Gulf of Maine, larval herring are routinely sampled with nets towed by vessels. We needed more information on the distribution and abundance of the larvae, but increasing the number of samples by towing was not feasible. We were also interested in exploratory sampling in areas inaccessible to towing and studied the application of stationary or buoyed and anchored nets, which utilize the "towing power" of tidal flows (Massman, 1952; Dovel, 1964). The design of the buoyed nets is described in this paper and catches made in 1963–65 are evaluated. We determined the straining or filtering efficiency of the nets, calculated the variation of catch rates for individual stations, and compared the seasonal changes in catch rates with those of towed gear.

DESIGN OF THE BUOYED AND ANCHORED NETS

The nets (Fig. 1) were buoyed at the surface and anchored to the bottom to sample at predetermined depths; flow meters provided a measure of sampling effort. The nets aligned in the direction of flow when fishing and closed when the tide was not flowing. Our design included four triangular wooden vanes, each attached at two corners to a buoyed and anchored line by snap-on connectors (Fig. 1A). A net with a mouth opening of 0.5-m diam was suspended by brackets fastened on each side of the free corner of each vane. The weight of a plastic sleeve connecting the net and codend was sufficient to collapse the net during slack water and prevent escape of the

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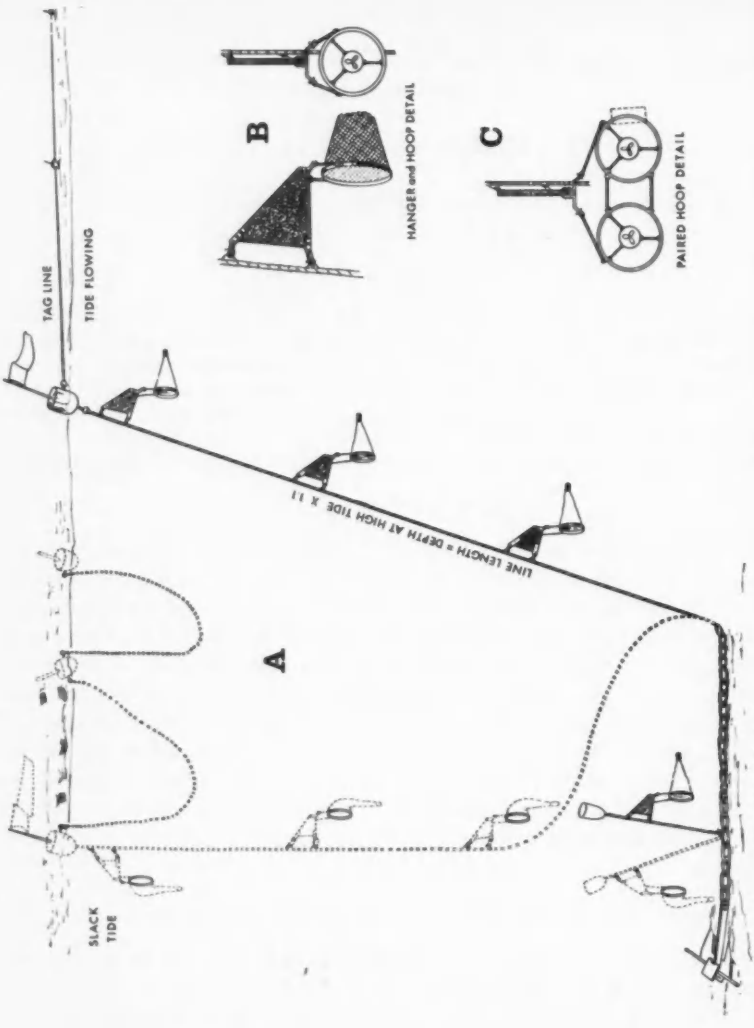

FIG. 1. Buoyed nets for sampling larval herring. A, positions of four nets during slack and flowing tides; B, arrangement of the hanger and hoop; and C, hoop arrangement for collecting paired samples, with attached board (broken line) used when determining the efficiencies of nets.

catch. When the tide flowed, the nets opened and the vanes aligned the nets in the direction of flow. Ropes attached to the upper sides of each hoop ensured that the mouth opening of the net faced the current. A Rigosha[3] flow meter was suspended at the center of the mouth of each net (Fig. 1B). The impeller of the meter required a minimal current of 3 cm/sec to revolve and the four

[3]Rigosha & Co., Ltd., Maruishi Building, Kajicho 1 chome, Kando Chiyoda-Ku, Tokyo, Japan. References to trade names in this publication do not imply endorsement of the products.

dials of the meter were capable of recording a total of 100,000 revolutions. One net was fixed near the buoy, two were near mid depth, and another was buoyed by a separate line and float near the anchor. If, when the nets were fishing, the height of the tide exceeded the length of mainline available, the anchor chain rose from the bottom but did not raise the net attached near the anchor. The surface and bottom nets fished at their predetermined positions; the nets near mid depth fished at constant distances below the surface but at varying levels in the water column according to the stage of the tide.

EFFICIENCY OF THE NETS

A measure of the volume of water filtered is needed to determine the straining efficiency of the nets. The flow meters were calibrated in meters per revolution of the impeller, over distances of 1.8 and 3.7 km at speeds of 103 cm/sec and 154 cm/sec. The unusually long distances were necessary to simulate the large amounts of water that would pass through the meters during a tidal cycle. The calibrations of individual meters varied from trial to trial for the two distances until we removed the binding coil spring from the impeller shaft. The spring had been installed by the manufacturer to disengage the impeller in periods of no flow. First estimates of the volumes of water strained for each flow meter (area of net mouth $\times$ meters per revolution) were from 0.0181 to 0.0214 m^3 per impeller revolution; 1 year later they varied from 0.0184 to 0.0217.

Straining efficiencies of the nets were determined by suspending flow meters in the hoops of the double frame (Fig. 1C). One hoop had a net attached; the other hoop had no net, but a small board was placed on the outside rim to equalize the drag and to help align the vane within the current. The meter with a net attached measured the rate (centimeters/second) of flow through the net, and the meter without a net measured the actual flow surrounding the net. The ratio of the two measurements provided an estimate of the efficiency of the net. Two methods, towing from a vessel and buoying and anchoring the nets in an estuary for 15-min periods, provided us with a range in current velocities expected in tidal waters.

We tested the straining properties of a monofilament mesh and two knitted nylon meshes after extensive use. Mesh apertures were 0.39, 0.74, and 1.02 mm, and the numbers of meshes per centimeter were 19, 9, and 6, respectively. The mesh aperture of the finer of the two knitted nets corresponded to the diameter of a 5-mm yolk-sac larval herring; and that of the coarser net to the diameter of a 20-mm larva. The efficiencies ranged from 80 to 90% at speeds above 30 cm/sec for the monofilament and above 15 cm/sec for knitted meshes; but efficiencies were extremely variable at slower speeds.

Currents less than 15 cm/sec are common in our tidal waters; therefore we investigated the source of these large variations within the smaller meshed (0.74 mm) knitted net. We observed the flow of water through nets suspended from a dock and Scuba divers observed the flow through nets anchored in a

channel. The same fluctuations in velocities that occurred within the mouth of the net were present in the surrounding water. They closely resembled the movements of eddies superimposed on an average flow, a characteristic of turbulent flow described by Alberston et al. (1960, p. 217). The similarities in fluctuations in velocities suggested that movement of water through the net was unimpeded at low velocities. Because the orientation and size of the eddies are random in turbulent flow, it would be unlikely that any systematic differences between the amounts of water moving through various portions of the mouth would persist through time (hours). It is suggested that the 15-min period during which the double hoop was buoyed and anchored in the estuary was insufficient to average the variations in velocity. The fluctuations in flow within the mouth of the net would cease when the average velocity (15 cm/sec) caused sufficient friction on the meshes of the net to "organize" the flow of water through the mouth. We concluded that during a flood or ebb tidal current a meter at the center of the net would give a reliable estimate of the total flow through the net.

Because the nets could capture large amounts of plankton over a tidal cycle, we tested the effect of clogged meshes on the efficiencies of the knitted and monofilament nets. Mesh openings were reduced for the tests (1) with plankton captured by the nets and (2) by artificially reducing the size of the cone of each net. The double hoop with one net attached was towed for 0.9, 2.8, and 3.7 km to coat the meshes with particulate (8–155 ml) and jellied (80–350 ml) plankton. Immediately after we collected the plankton the double hoop was towed first at 156 and then 200 cm/sec for 15 min. The nets were then cleaned, one third of each cone was tied off to simulate clogging and the tows repeated for the two speeds. The efficiencies of the knitted nets were not affected; the efficiency of the monofilament net was 80% when the size of the cone was reduced and dropped to 68% when the meshes were clogged.

EXPLORATORY SAMPLING OF 1963–64

Sets of buoyed nets were made to determine whether larval herring could be captured in locations difficult to reach with towed nets. In the autumn of 1963, before our experiments with knitted nets, we began trials in the Sheepscot estuary with buoyed monofilament (aperture 0.39 mm) plankton nets without meters. These sets captured larval herring. During the winter, sets were made in other coastal areas that were difficult to sample with towed gear. The nets captured larval herring in five of the seven estuaries (Fig. 2). The absence of larval herring in catches from estuaries of the Machias and Pleasant rivers during the winter coincided with an absence of larvae in catches of towed gear in the bays adjacent to the mouths of the estuaries.

In the summer of 1964 we made a total of twelve 12-hr sets in the Damariscotta estuary. Paired and metered knitted nets were mounted on the double hoops, at the surface and at 10 m. The nets, 12 pairs at each depth, captured zooplankton and larval fishes other than herring, which are not

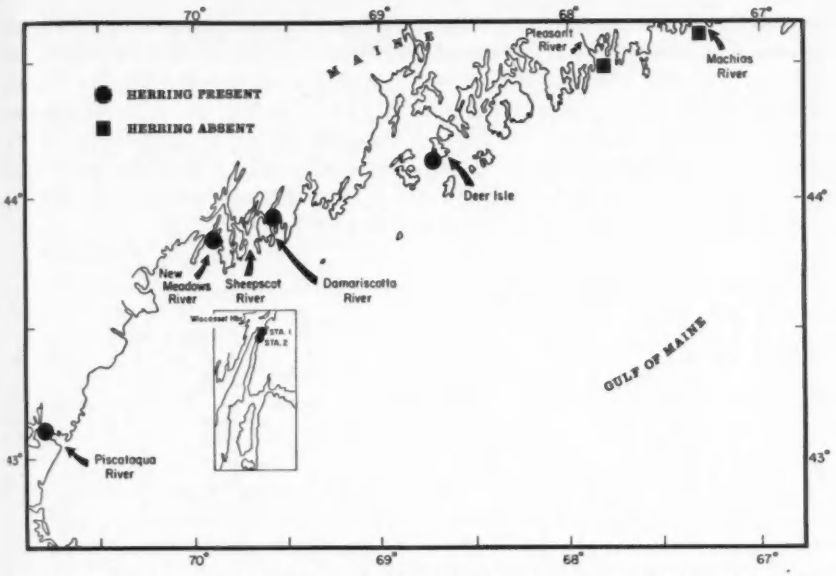

FIG. 2. Exploratory sets of buoyed nets for larval herring along the coast of the Gulf of Maine during the winter, 1963–64.

present in the summer. For each net we counted the fish, measured the displacement volume of zooplankton, and calculated the volume of water strained. The number of larval fish averaged 20 and varied from 1 to 162. Volumes of plankton averaged 36.2 ml and varied from 2 to 90 ml. The amounts of water strained by all of the paired nets differed greatly. But statistical comparison (*t*-test) of catches per 100 m^3 of water strained showed no significant differences for plankton volumes ($P > 0.50$) or larvae ($P > 0.35$) among the 24 catches from both depths.

SAMPLING IN SHEEPSCOT ESTUARY, 1965

CATCH RATES AT TWO STATIONS

In the spring of 1965 we captured larval herring by fishing serially a set of four knitted nets at each of two stations for five consecutive nights in the Sheepscot estuary (Fig. 2). Sufficient distance was allowed between the sets along the estuarine channel to prevent tangling. The channel was 370 m wide and 20 m deep and larval herring were present throughout the experiment. We set the nets and fished them until the next slack tide. During slack tide the nets were retrieved, reset, and a hydrographic cast was made between the stations. The nets were retrieved again at dawn. Ebb tides were fished for $2\frac{1}{4}$–$5\frac{1}{4}$ hr and flood tides for $5\frac{1}{2}$–$6\frac{1}{2}$ hr. The 20 sets (2 each night at each station) captured 14,312 larval fish, including 4025 larval herring. The catches of individual sets varied from 49 to 1002 for all larval fish and from 21 to 444

for herring. Catch per unit effort was calculated for each set of four nets from the total amounts of water strained and total catches of all larval fish and herring (Fig. 3). The amount of water strained increased with the length of time fished and an F-test suggested that the relations for the two stations fitted a common slope ($P > 0.20$). This evidence was sufficient to justify grouping the data from sets at the two stations in pairs and to compare them statistically by a *t*-test. Most catch rates at station 1 were greater ($P < 0.01$) than those at station 2 for all larval fish and for herring.

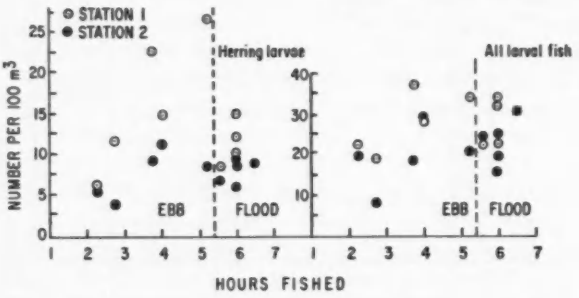

FIG. 3. The relations of the catches of larval fish at two stations to the numbers of hours fished during ebb and flood tides.

EFFECT OF TIDAL EXCURSIONS ON CATCH RATES

The differences between catch rates at stations 1 and 2 were related to changes in tidal current and the distribution of larvae. To examine currents we calculated tidal excursions[4] from the readings of flow meters in each net (flow meter reading × calibration factor in meters/revolution of impeller). Vertical profiles of the excursions were usually typical of those expected in estuaries; excursions were usually longest at 10–15 m on the flood tide and at the surface during ebb tide (Fig. 4). The profiles were similar for the two stations until the fourth and fifth nights of fishing, when they became atypical during the flood tide at station 1. These atypical conditions in the profiles were associated with increased river discharge shown by lowered salinity and increased temperature (Table I). Station 1 abutted a large embayment, which apparently made it more sensitive to alterations of the tidal currents from the addition of fresh water to the estuary.

The depth distributions of catches of larval fish resembled the distributions of tidal excursions. Vertical distributions of larval herring (Fig. 5) frequently showed the greatest numbers of larvae at 10 and 15 m for flood tide and at the surface for ebb tide. The distribution (percentage) of larvae at each depth was correlated with the excursion for both tides at station 2 (flood $r = 0.88$,

[4]A tidal excursion is the distance a given particle of water moves away from the position of the anchored net during a flood or ebb tide.

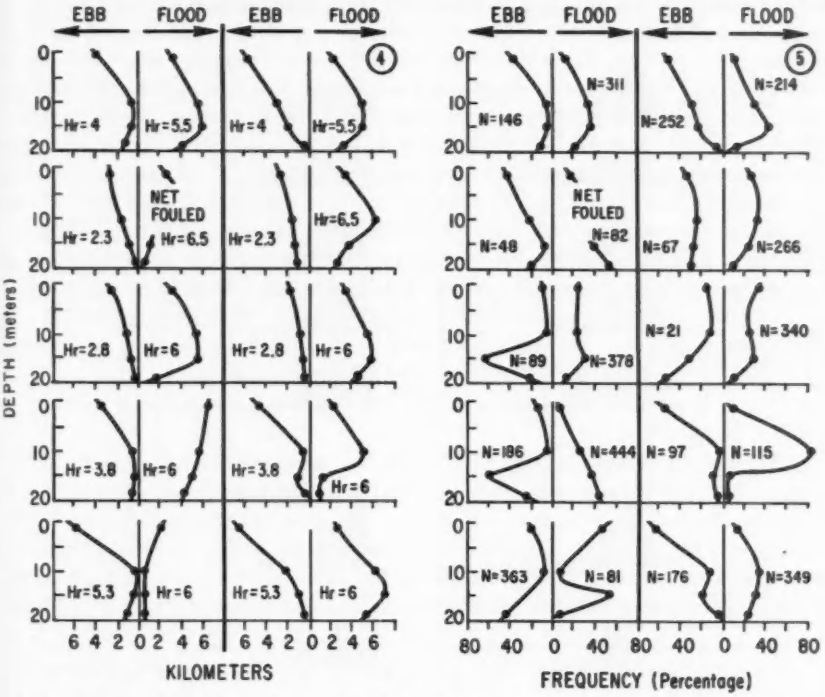

FIG. 4. Profiles of tidal excursions at stations 1 (*left*) and 2 (*right*) in the Sheepscot estuary, arranged in order of consecutive nights of fishing, April 5 (*top*) to April 9 (*bottom*), 1965.

FIG. 5. Depth distributions of larval herring at stations 1 (*left*) and 2 (*right*) in the Sheepscot estuary, arranged in order of consecutive nights of fishing, April 5 (*top*) to April 9 (*bottom*), 1965.

TABLE I. Water temperatures, salinities, and catch rates (numbers/100 m³) of larval herring (ebb and flood tides combined) for the Sheepscot estuary, April 5–9, 1965.

	Temp (C)								
		Bathythermograph			Salinity (‰)			Catch rates	
Night of operation	Bucket Surface	0 m	10 m	15 m	0 m	10 m	15 m	Station 1	Station 2
1	3.4	3.5	3.8	3.7	29.9	30.5	30.7	9.6	8.7
2	3.4	3.4	3.3	3.1	30.0	30.4	30.8	8.4	8.8
3	4.4	4.3	4.1	3.9	28.3	29.1	29.5	12.2	7.9
4	4.4	4.1	4.2	4.2	28.6	28.9	29.2	12.4	6.6
5	4.5	–	–	–	27.3	28.6	29.3	23.6	8.8

ebb $r = 0.86$, df = 18, $P_{.05} = 0.56$) and for the flood tide at station 1 ($r = 0.75$, df = 14, $P_{.01} = 0.62$). The correlation for data from the ebb tide at station 1 ($r = 0.49$, df = 18, $P_{.05} = 0.44$) was borderline; the numbers of larval herring were large in short tidal excursions on the 3rd–5th nights. This association produced large ratios of catch to volume (m^3) of water strained (42/4.7, 15/2.7, and 97/0.2) during the 3rd–5th night of fishing, when temperature increased and salinity decreased progressively in response to increased river discharge into the estuary. Catch rates at station 1 varied with changes in temperature and salinity but those at station 2 did not (Table I). Perhaps the larger catch rates at station 1 represented an accumulation of larvae during daylight in areas of low tidal velocities where they could await a favorable tide. Creutzberg (1961) described an analogous situation for elvers that move shoreward on the flood tide and then seek bottom to prevent their transport seaward during the subsequent ebb tide.

Sizes of Larvae

The length distributions of larval herring were similar at the two stations. Length frequency plots at station 1 varied little from those at station 2 for a given depth and their modes tended to occur at a slightly larger size at the surface and bottom than at midwater at both stations (Fig. 6). Further, when the data were combined for all depths, the differences in the configurations of the plots between flood and ebb tides were similar in detail at the two stations.

SEASONAL CHANGES IN CATCH RATE IN THE SHEEPSCOT ESTUARY

Larval herring hatch at 5 mm (standard length) in the autumn and metamorphose at 45 mm in the spring. In 1964–65, we towed a 1-m net in the Sheepscot and Damariscotta estuaries (Fig. 2), but it was effective only in the autumn (Graham and Boyar, 1966). We also towed a Gulf III sampler from the period of hatching until the larvae reached a length of 20 mm, and then used an especially designed trawl — the Boothbay depressor trawl (Graham and Vaughan, 1966) — for larger larvae.

From October 1964 through April 1965 we made a series of nighttime sets of buoyed nets in the narrow channel of the Sheepscot estuary, and compared the catches with those obtained concurrently with the gears that were towed within the surrounding area (Fig. 7).

The stationary and towed gears showed the same seasonal increase in lengths of larvae. Catch rates were highest in the autumn, lowest in the winter, and high again in the spring. Catches with towed gears, mostly made during daylight, were much smaller than those made at night with the buoyed nets. Catch rates from individual night tows near stations 1 and 2 in the Sheepscot (Fig. 2) were more comparable with those of the buoyed nets. On March 16, 12.9 larval herring per 100 m^3 were captured with the trawl at night and 56.0 on April 27. During the intervening period April 5–9, individual sets of buoyed nets captured from 3.7 to 27.1 larvae per 100 m^3.

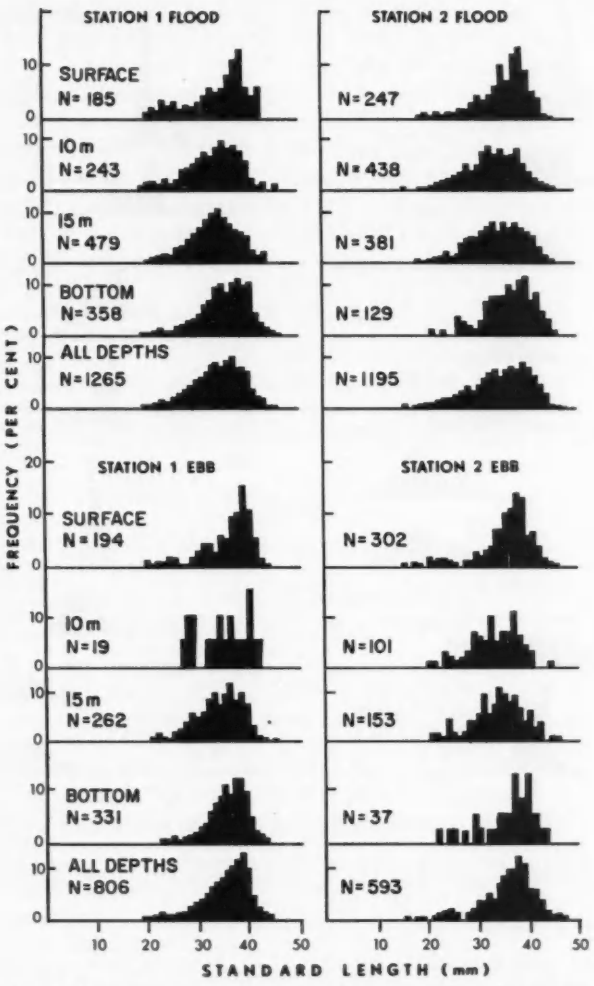

FIG. 6. Length frequencies of larval herring captured during the flood and ebb tides at stations 1 and 2 in the Sheepscot estuary (data combined for five consecutive nights of fishing, April 5–9, 1965).

COMPARISON OF BUOYED AND TOWED NETS AS SAMPLING GEAR

Differences between day and night catches, which are common for herring, result from avoidance of the towed gears by the larvae during the day and their redistribution vertically from day to night (Colton et al., 1961; Dragesund, 1965). The relation between the vertical distribution of the larvae and the

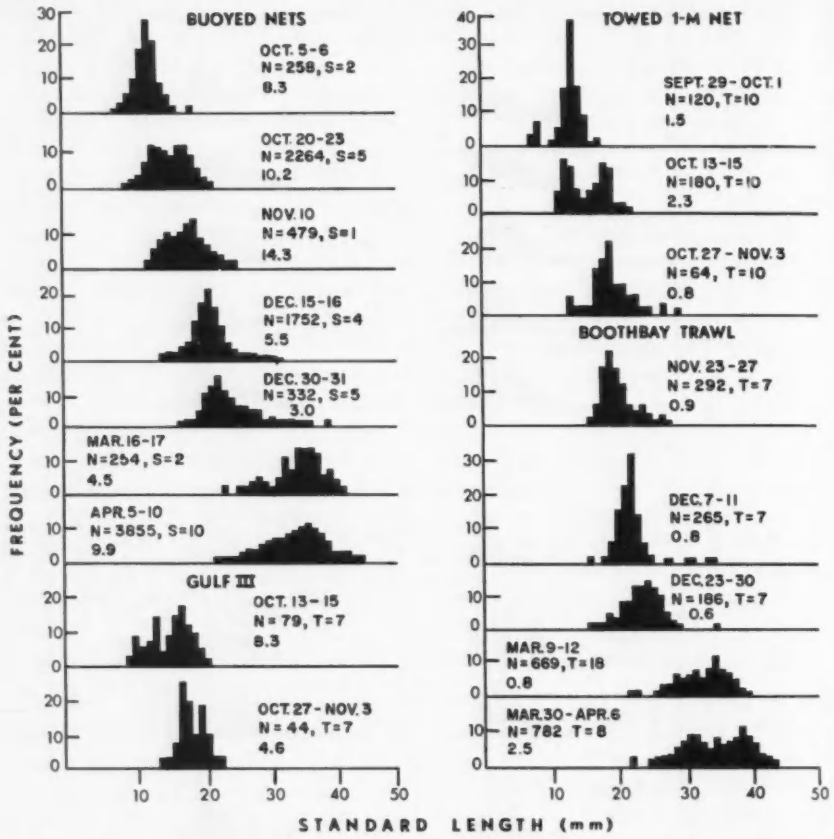

FIG. 7. Comparison of catches of larval herring in buoyed nets with catches in towed gears. N, number of fish; T, number of tows; S, number of sets; and 0.0, catch per 100 m^3.

lengths of the tidal excursions for many of the night sets suggests that (1) larvae were evenly dispersed along the channel of the estuary and (2) avoidance of the net or escapement of larvae was small before the onset of metamorphosis. But avoidance or escapement did apparently occur during daylight. Only one or two larval herring per set were captured in daylight with the buoyed nets, whereas several hundred were taken at night. During the day, the towed gears captured larvae of the same size-range as those captured at night by the buoyed nets. This success in capturing larvae, especially in the spring when they were large, attested to the advantage of the trawl in daytime, and probably was due to a high towing velocity of 206 cm/sec and a larger mouth opening (1 m^2) than that of the buoyed nets.

Towed and stationary gear both have limitations when used to detect changes in the distribution of larval herring over brief periods of time. Hopkins (1963) reported that several tows are necessary at a given station to obtain a representative sample from a single tide with a Clarke–Bumpus sampler. Thus, to sample at a series of stations several times during a tidal cycle requires considerable effort. Since our trawl tows cover about 3.7 km and last 30 min, only a few stations can be sampled during a tide. Buoyed nets can be set to fish "synoptically" over an entire tidal cycle, but the volume of water filtered by buoyed nets is sometimes very small and not comparable with the larger volumes sampled by towed gear.

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Subdivisions of the Benthic Environment of the Upper Great Lakes, with Emphasis on Lake Michigan[1,2]

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ABSTRACT

An effort has been made to introduce a greater degree of standardization and reliability into descriptions of the benthic environment made by biologically oriented observers. Comparisons of field descriptions and mechanical analyses showed an encouraging ability to distinguish among four major sediment types in Lake Michigan. The bottom of the lake has been described in terms of these types, which usually occur in the following order with respect to depth: sand, silty sand–sandy silt, silt–clayey silt in a thin layer overlying stiff plastic clay, and silt–clayey silt only. Each sediment type tends to exhibit its own range of organic carbon values, with the amount of carbon increasing with depth. Hard or rocky bottom occurs in more restricted areas and is considered a fifth recognizable bottom category. Certain features of the distribution of the sediments may be related to gross water movements and to the locations of the mouths of large tributary rivers. Limited comparative studies in Lakes Huron and Superior showed similar distributions of sediment types and organic carbon content. It is suggested that the observed uniformity of maximum organic carbon values in the sediments of all three lakes may be related to processes of oxidation.

INTRODUCTION

Studies of the benthic environment in the upper Great Lakes have increased markedly over the past decade. Whereas the only thorough study of the macrobenthos in the upper lakes prior to World War II was that of Eggleton (1936, 1937) in Lake Michigan, since that time, and especially since 1960, a number of workers have investigated various aspects of the macrobenthos. For Lake Michigan, Merna (1960) presented data relating to the geographic and topographic distribution of numbers of benthic organisms; Cook and Powers (1964) made an intensive study of the macrobenthos in the immediate vicinity of the St. Joseph River and attempted to relate the effect of the river outflow to the faunal distribution; Powers and Robertson (1965) investigated the areal and depth distribution of the macrobenthos in the southern two-thirds of the lake with respect to numbers and kinds of organisms and ash-free dry weight; and Robertson and Alley (1966) compared the quantity of macrobenthos in the lake in 1964 with that found by Eggleton in the early 1930's. For Lake Huron, Teter (1960) presented data on the numbers and kinds of macrobenthic organisms and their relation to depth; Henson and Herrington (1965) carried out a taxonomic and distributional study of the

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sphaeriids in the Straits of Mackinac region; and Schuytema and Powers (1966) studied the distribution of numbers of organisms in Lake Huron and compared this with results from Lake Michigan.

Continuing studies of the benthic environment of Lake Michigan were initiated by us at the Great Lakes Research Division in 1964. It has become increasingly obvious that benthic productivity is influenced by a complex of environmental factors, and attention has been directed toward various aspects of the complex. One major aspect is the influence of sediments. Emphasis has been upon the identification of sediment types; their distribution from the standpoints of depth, bottom topography, and geographical location; and the quantity of organic matter which they contain. The present paper presents some of the interrelationships among sediment type, organic carbon, and water depth which have become apparent. This work has permitted the definition of rather discrete benthic zones, whose biological significance will be treated in a future paper.

MATERIALS AND METHODS

The benthic environment of Lake Michigan was studied on a monthly basis, except for the winter months, at 34 stations located in the southern two-thirds of the lake (solid circles, Fig. 1). These stations were visited on an average of 15 times from August 1964 through June 1966. Triplicate grab samples of the bottom sediments were obtained during each station visit for studies on the macrobenthos (Powers and Robertson, 1965) and for identification of sediment type.

Samples were taken with a Smith–McIntyre dredge (McIntyre, 1954) until June 1965 and a Ponar grab sampler (Powers and Robertson, 1967) thereafter. The grab was opened into a large washtub and the sediment carefully transferred to the tub in an undisturbed condition. The sample was described with respect to apparent sand, silt, and clay content as determined visually and tactilely; inclusions such as granules, pebbles, and cinders were noted; and all visible layers were described individually. Sediment color was estimated and recorded. This resulted in such descriptions as "clean medium sand," "fine brown silty sand," "gray silt over tan plastic clay," and the like.

Ayers and Hough (1964) have published field descriptions of sediments from Lake Michigan which we have used to supplement the observations from our own 34 stations. Their samples were taken on a number of sampling lines which are shown in Fig. 1. These lines were roughly perpendicular to shore and from 5 to 15 miles apart. On each line samples were usually taken as follows: at 1-mile intervals from the 1st through the 10th mile from the beach, at 2-mile intervals from the 10th through the 20th mile, and at 5-mile intervals thereafter. A single grab sample of the surficial sediment was obtained at each station and described in the same manner as outlined above.

Particle size analyses were not performed on our samples, but they have been made on those samples of Ayers and Hough which were taken in the

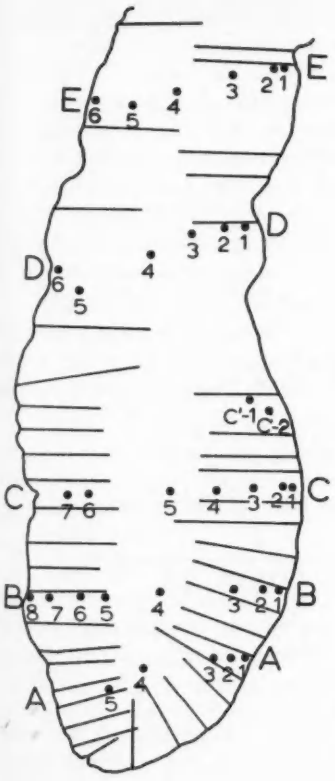

FIG. 1. Locations of stations and sampling lines, Lake Michigan.

southern basin, south of the Milwaukee–Muskegon region. Analyses for the west half of the lake were made by Paul D. Josephson, Great Lakes Research Division (personal communication), and from the east half by Cote (1967). Using the percentages of sand, silt, and clay (defined with respect to particle diameter according to the Wentworth scale) as obtained from the results of these analyses, we have assigned soil-type nomenclature to the Ayers and Hough samples. This was done following the method of Shepard and Moore (1955) as used by Lauff et al. (1961) in studies of the Straits of Mackinac region. Each sample was plotted on a triangular graph (Fig. 2) with respect to its percentage of sand, silt, and clay, and nomenclature assigned according to the section of the graph in which the point fell. The following sediment types were defined: sand, clayey sand, silty sand, sandy silt, silt, clayey silt, and sand–silt–clay (loam). In addition, at a number of stations off Milwaukee–Racine and Waukegan–Michigan City hard bottom was encountered which was composed variously of gravel, cobbles, and hard till, frequently interspersed with small amounts of sand.

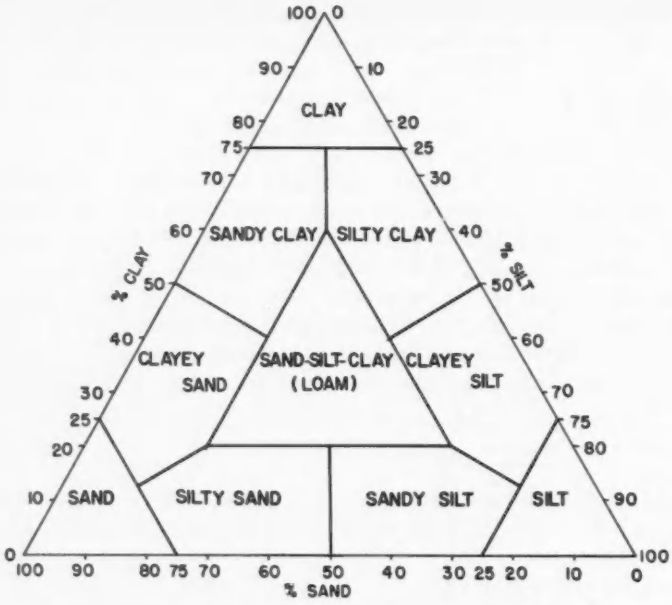

FIG. 2. Triangular graph for assigning sediment nomenclature based on percentage sand, silt, and clay.

The descriptions based on laboratory analyses were compared with the corresponding field descriptions. Further, since most of our southern basin stations were located quite near at least one of the Ayers–Hough sampling lines, it was also possible to compare the field descriptions from our own stations with laboratory-based descriptions from their nearby stations.

After comparing the results of the two methods of sediment description, it was, not surprisingly, concluded that field examination does not produce descriptions of sediment type with the precision and reliability of the triangular graph method which is based upon laboratory analysis. Considerable confusion was evident in attempts to differentiate visually among silty sand, sandy silt, and clayey sand, and between silt and clayey silt. Sand–silt–clay (loam) was not a recognizable sediment type in the field and appears in the results of the laboratory analyses where layered samples of silt or clayey silt over stiff sandy clay were processed without respect to the layering.

The inability to distinguish in the field among the more subtle variations was resolved by simply combining those similar types which were frequently confused. This resulted in the definition of five categories of lake bottom which, although rather broadly based, appear to be recognizable with a good degree of consistency in field descriptions: sand, silty sand–sandy silt, silt–clayey silt, a layering of silt–clayey silt overlying stiff plastic clay, and the

various types of hard bottom. The category silty sand–sandy silt includes clayey sand (which appeared only twice in the samples.) In the layered sediment the upper silt–clayey silt stratum varies in thickness from about $\frac{1}{16}$ inch to about 1 inch. The underlying clay may be sandy to various degrees, and grayish, brownish, or salmon-pink in color. Silty sand–sandy silt is readily distinguished from silt–clayey silt because of its greater firmness and gritty texture. Stiff clay, a major component of layered sediment, is of course easily identified, but the noncompacted clays, usually gray and quite soft and soupy, are very difficult to separate visually from silt; hence the lumping of the noncompacted clays with silt.

For simplicity of presentation, the categories of sediment types as just defined will hereafter be designated as follows: sand = sand; silty sand–sandy silt = silty sand; silt–clayey silt = silt; silt–clayey silt overlying stiff plastic clay = layered; gravel, cobbles, till, etc. = hard.

Sediment samples for the determination of organic carbon were taken in triplicate at each of the 34 stations in July and October 1965 and in April 1966 to obtain estimates on a seasonal basis. A short gravity corer of 2-inch (5-cm) inside diam was used in July, and a Ponar grab sampler the other 2 months.

The corer was designed to accept removable plastic liners so that extrusion of cores in the field was not necessary. Each liner, while being maintained in a vertical position, was removed from the core barrel and quickly corked at the bottom. The core was left upright for a sufficient time to permit resettling of the surficial sediment, after which the supernatant water was drained by drilling a small hole through the liner a short distance above the top of the sediment. A second cork was then pushed down to the top of the sediment. The excess liner was cut off, and the core immediately frozen at about −20 C.

Initially it was thought that the corer would obtain a more representative, more nearly undisturbed section of the sediment–water interface zone than the grab sampler, particularly since the sediment would be contained in a plastic core liner which would eliminate the need for subsampling. However, the use of a corer is a tedious, time-consuming technique, and accurate removal of a representative sample from the top, after thawing in the laboratory, was difficult owing to the slumping of the sediment. It was therefore decided to attempt the October sampling with a Ponar grab. Triplicate samples were taken at each station, and a subsample removed with a spatula from the top 2 cm of each. In the case of layered sediment, only the top layer was subsampled. Each subsample was sealed in a small plastic bag and frozen. The April samples were taken in the same manner.

Determinations of organic carbon followed the "wet oxidation" method of Grewling and Peech (1960). Dried and weighed sediment samples were digested with a mixture of potassium dichromate and concentrated sulfuric acid. Calculations of per cent organic carbon were based on the quantity of

dichromate reduced as determined by titration with ferrous sulfate, according to the relationship

$$\text{per cent C} = \frac{4(\text{volume of 1 N dichromate reduced})\ (100)}{\text{weight of sample in milligrams}}$$

where 1 ml of 1 N dichromate = 4 mg C. As will be shown later, no differences in organic carbon content were found between samples taken with the corer and those taken with the Ponar.

RESULTS

Distribution of Bottom Sediments

The bathymetry of the lake (Fig. 3) shows a definite tendency for a division into two basins, separated by a partial sill extending roughly between

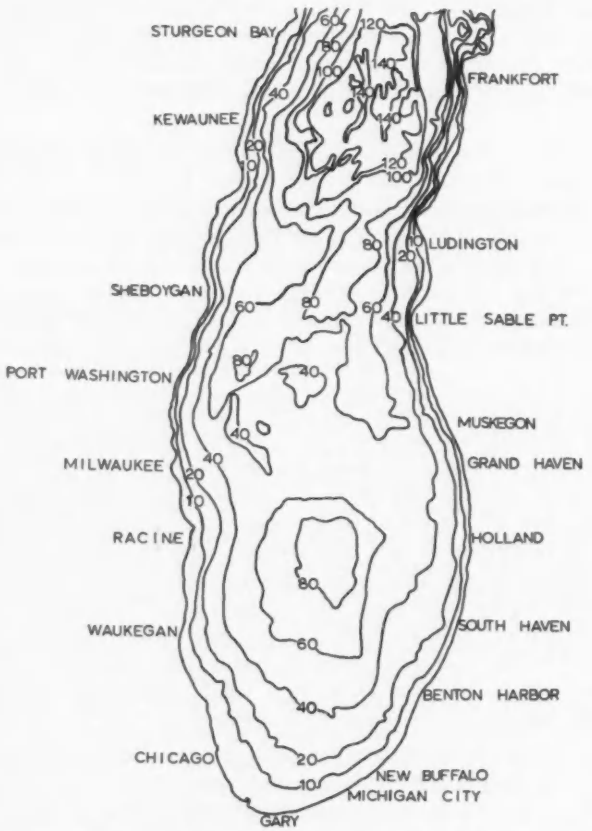

Fig. 3. Orientation map and bathymetry of Lake Michigan. Depths in fathoms.

Muskegon on the east side to Milwaukee–Port Washington on the west. This sill is shallowest in midlake where it shoals to about 44 m (24 fath). The sill does not extend continuously to either shore, being interrupted by relatively deep channels on both sides of the lake. A portion of the west end of the sill is known to consist of rocky escarpments.

The locations of the stations on the Ayers–Hough sampling lines and of our 34 stations were plotted on a navigation chart of Lake Michigan. The corresponding sediment type, expressed as one of the five basic categories described in the preceding section, was entered at each station location. From the resulting distribution of known sediment types the major boundaries of sand, silty sand, silt, layered sediment, and hard were interpolated to produce the sediment map shown in Fig. 4. Precise location of boundaries was not, of course, always possible, but enough data points existed to permit reasonable estimation. Data points for the northern basin of the lake were considerably less dense than for the southern basin, and the boundaries north of the sill are subject to a greater degree of uncertainty.

Inspection of the sediment map shows that sand and silty sand are nearshore, shallow water sediments restricted to narrow bands extending almost continuously around the shores of the study area. The continuity is interrupted in the two areas of hard bottom off Milwaukee–Racine and Waukegan–Michigan City, and between Ludington and Little Sable Point where silty sand is not found.

The predominant bottom types in the lake are layered sediment and silt. The relative distribution of the two sediment types is not the same in the northern and southern basins. It can be seen from Fig. 4 that north of the sill layered sediment occupies a greater area of lake bottom than does silt, which appears to be restricted to the deep basins and channels in that part of the lake. Most of the western part of the northern basin consists of layered bottom, except for the shoreward bands of sand and silty sand and a restricted area of silt occupying an arc-shaped trench between Sheboygan and Milwaukee. Silt occurs in the very deep water between Frankfort and Kewaunee, where the deepest soundings in Lake Michigan (up to 282 m; 154 fath) are found, and is continuous into the southern basin through the channel which interrupts the sill and extends down the eastern side of the lake. A narrow band of layered sediment lies between the silt zone and the sands off the eastern shore between Frankfort and Little Sable Point.

Whereas silt in the northern basin is found only in deep water, south of the sill it extends in shoreward of the 20-fath (37-m) depth contour. The isobaths in the southern basin tend to describe a lopsided saucer (Fig. 2) having its greatest depth in mid lake between Holland and Racine. The distribution of silt, however, does not conform to this topography. Offshore from the narrow coastal strips of sand and silty sand, silt occupies the eastern one-third of the basin as far south as Benton Harbor. Between, roughly, Holland and South Haven, the silt region extends into and beyond mid lake to occupy the deeper portion of the basin.

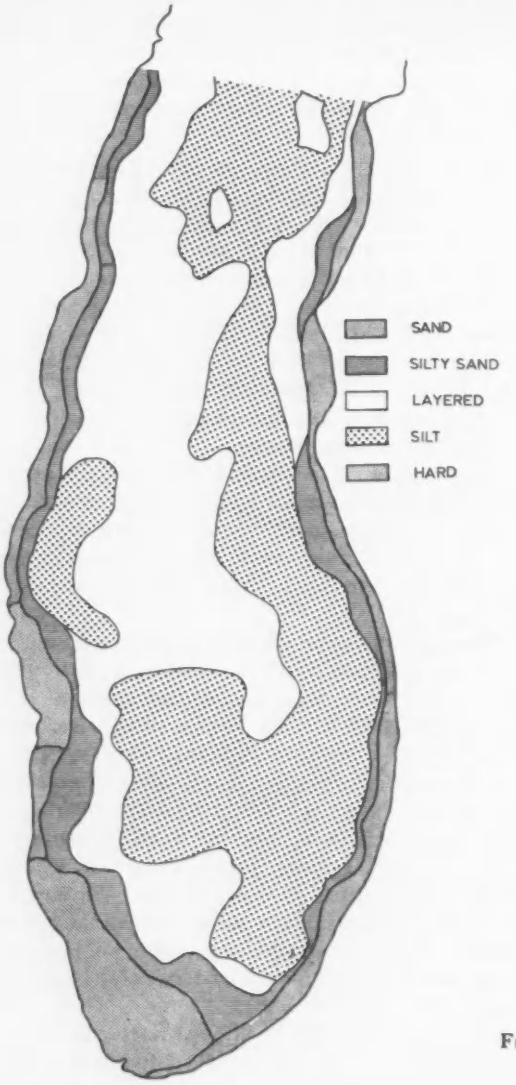

FIG. 4. Distribution of sediment types in Lake Michigan.

Layered sediment is greatly restricted in the southern part of the lake, appearing as a narrow band which extends from the northern basin down the western side of the lake until, off Waukegan, it turns southeast and terminates off New Buffalo. Layered sediment also protrudes across the sill in the east-central part of the lake off Holland, but extends only a short distance into the southern basin.

Silty sand on the west and south sides of the southern basin lies farther offshore, for the most part, than anywhere else in the lake. It is separated from the shore by extensive areas of hard bottom between Milwaukee and Michigan City except for a short stretch of sand between Racine and Waukegan. In this latter region the silty sand belt approaches shore more closely than off the areas of hard bottom.

Organic Matter in the Sediments

Organic carbon determinations were made on sediment samples taken at our 34 stations as a measure of the quantity of organic matter incorporated into the surficial bottom deposits of the lake. Triplicate samples were taken at each station in July, October, and April and analyzed for carbon in order to ascertain whether significant seasonal differences occurred. The results, as averages of the triplicates, appear in Table I.

A Friedman two-way analysis of variance by rank test (Siegel, 1956) was performed on the three sets of data to determine whether the percentage of organic carbon varied seasonally. No differences were found at the 5% significance level ($x^2r = 0.26$, N = 31, df = 2, $p > 0.80$).

The interrelations of sediment type, organic carbon, and depth as observed in the above 3 months are shown in Fig. 5. It is evident that organic carbon generally increases with depth, but the increase is not uniform and is influenced by sediment type. The smallest quantities of organic carbon occur in sand, which was sampled only in shallow water at depths of 44 m (24 fath) and less and exhibited carbon values ranging between 0.04 and 0.22%. Silty sand overlapped slightly in both depth and organic carbon content with sand; it was found between 35 and 93 m (19 and 51 fath) and ranged in percentage organic carbon from 0.16 to 0.71%. Layered sediment usually occurs in intermediate depths; among our 34 stations it was found between 74 and 132 m (40 and 72 fath), and the organic carbon values in its upper, silt layer ranged from 0.66 to 1.69%. However, in July and April it was also found at station E-4 at a depth of 216 m (118 fath), and there the average organic carbon content of the upper layer averaged 2.72%. Silt, which is found in the deepest parts of the lake, was also found as shallow as 47 m (26 fath) at station B-2 off South Haven, and thus completely overlaps the depth range of the layered sediment. At depths over 132 m (72 fath) it was the only bottom type encountered, except for station E-4. The organic carbon content of silt is comparatively high, ranging from about 1.70% at station B-2 in 47 m (26 fath) to 4.02% at station E-5 in 173 m (95 fath). The percentages of organic carbon in silt from depths greater than 170 m (93 fath) were the highest found.

Comparative Studies in Lakes Superior and Huron

We obtained a limited amount of data in July 1966 on the sediments and their organic carbon content in Lakes Superior and Huron. Methodology was the same as that described for Lake Michigan. Triplicate Ponar samples

TABLE I. Relation of sediment types, organic carbon, and depth in Lake Michigan: July, October 1965; April 1966. Positions of the stations appear in Fig. 1.

Station	Depth (m)	Location		Per cent organic carbon				Sediment type
		N lat	W long	July	October	April	Avg	
A-1	18	42°06′30″	86°32′00″	0.08	0.04	0.01	0.04	Sand
A-2	35	42°06′00″	86°37′00″	0.96	0.64	0.53	0.71	Silty sand
A-3	70	42°05′30″	86°43′00″	1.83	1.82	1.74	1.80	Silt
A-4	74	42°03′30″	87°06′30″	1.05	2.90	1.13	1.69	Layered
A-5	43	41°57′00″	87°18′30″	0.22	0.10	0.17	0.16	Silty sand
B-1	19	42°24′00″	86°20′30″	0.30	0.05	0.02	0.12	Sand
B-2	47	42°24′00″	86°27′00″	1.72	1.66	1.73	1.70	Silt
B-3	68	42°24′00″	86°35′30″	2.78	2.22	3.12	2.71	"
B-4	129	42°23′30″	87°01′30″	2.57	3.11	3.34	3.01	"
B-5	108	42°22′30″	87°21′00″	2.20	2.40	2.88	2.49	"
B-6	83	42°22′30″	87°30″00′	0.96	1.26	1.54	1.25	Layered
B-7	45	42°22′00″	87°40′00″	0.19	0.44	0.47	0.37	Silty sand
B-8	11	42°22′00″	87°47′30″	0.09	0.15	0.13	0.12	Sand
C-1	20	42°49′40″	86°14′50″	0.23	0.07	0.03	0.11	Sand
C-2	50	42°49′40″	86°18′25″	2.20	1.60	1.34	1.71	Silt
C-3	77	42°49′10″	86°28′25″	2.50	3.11	2.73	2.78	Silt
C-4	108	42°48′50″	86°41′30″	1.22	1.12	2.51	1.62	Layered
C-5	157	42°49′00″	86°50′00″	2.13	3.17	3.76	3.02	Silt
C-6	99	42°47′40″	87°26′50″	0.72	0.64	0.61	0.66	Layered
C-7	55	42°47′30″	87°34′30″	0.29	0.27	0.23	0.26	Silty sand
C′-1	38	43°08′00″	86°23′00″	0.53	0.46	–	0.50	" "
C′-2	93	43°12′00″	86°31′00″	0.61	0.44	–	0.52	" "
D-1	30	43°57′00″	86°33′00″	0.07	0.11	0.03	0.07	Sand
D-2	98	43°56′00″	86°39′30″	0.74	1.15	0.81	0.90	Layered
D-3	170	43°54′00″	86°51′30″	–	3.60	3.43	3.52	Silt
D-4	131	43°48′00″	87°03′00″	0.72	0.79	0.75	0.75	Layered
D-5	119	43°38′40″	87°31′00″	0.61	0.60	0.83	0.68	Layered
D-6	30	43°44′00″	87°38′00″	0.24	0.19	0.20	0.21	Sand
E-1	44	44°37′30″	86°18′12″	0.50	0.10	0.05	0.22	Sand
E-2	197	44°37′00″	86°21′42″	3.73	3.08	3.07	3.29	Silt
E-3	271	44°34′00″	86°40′00″	3.47	3.31	3.86	3.55	Silt
E-4	216	44°30′18″	86°55′18″	2.21	–	3.24	2.72	Layered
E-4	216	–	–	–	3.72	–	3.72	Silt
E-5	173	44°25′30″	87°10′18″	4.07	3.91	4.08	4.02	Silt
E-6	33	44°27′48″	87°26′25″	0.27	0.19	0.14	0.20	Sand

were taken at 10 stations in northern Lake Huron and at 7 stations in eastern Lake Superior (Fig. 6). Station 1 in Huron and station 3 in Superior were in areas of rocky bottom and samples for organic carbon determinations were not obtained. Seven of the Lake Michigan stations sampled previously

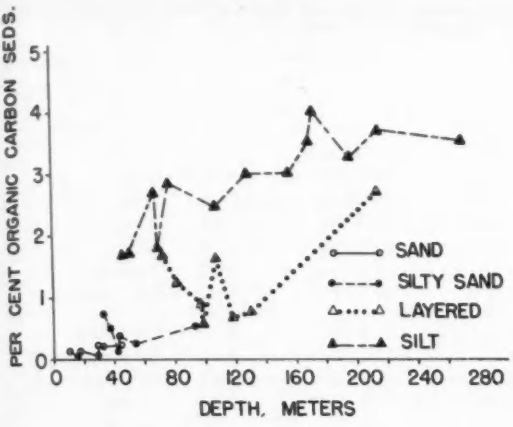

FIG. 5. Interrelations of sediment type, organic carbon, and depth in Lake Michigan.

(stations C-1 through C-7) were resampled at the same time for comparative purposes. The July 1966 observations for all three lakes are summarized in Table II. Per cent organic carbon as given is the average of triplicate samples, with two exceptions. Station SU-4 was over a very steep slope and the three bottom sediment samples obtained there were from highly variable depths; data for the three samples have not been averaged and are listed individually.

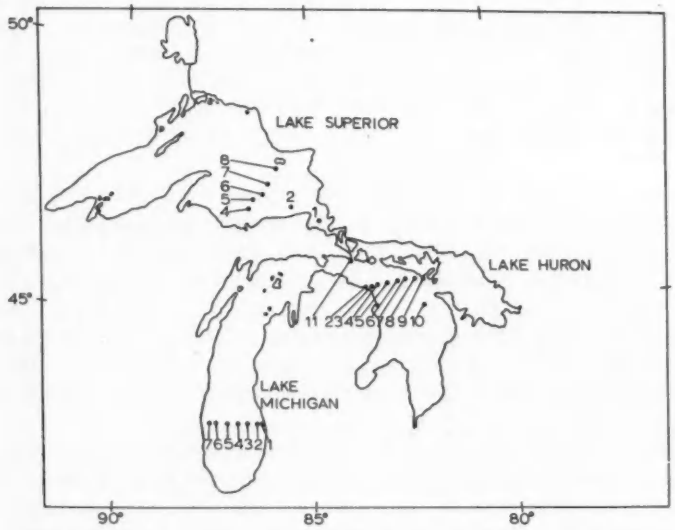

FIG. 6. Locations of stations in Lakes Michigan, Huron, and Superior, July 1966.

TABLE II. Relation of sediment types, organic carbon, and depth in Lakes Michigan, Huron, and Superior. July 1966.

Station	Depth (*m*)	N lat	W long	% organic C	Sediment type
C-1	25	42°49′40″	86°14′50″	0.05	Sand
C-2	53	42°49′40″	86°18′25″	1.33	Silt
C-3	80	42°49′10″	86°28′25″	1.84	Silt
C-4	100	42°48′50″	86°41′30″	2.06	Layered
C-5	162	42°49′00″	86°50′00″	3.44	Silt
C-6	100	42°47′40″	87°26′50″	0.77	Layered
C-7	54	42°47′30″	87°34′30″	0.24	Silty sand
HU-2	21	45°25′12″	83°41′48″	0.02	Sand/clay
HU-3	65	45°25′48″	83°31′12″	0.42	Silty sand/clay
HU-4	102	45°27′00″	83°12′12″	0.82	Silty sand/clay
HU-5	86	45°27′54″	82°56′48″	2.27	Layered
HU-6	141	45°28′42″	82°43′18″	1.70	Layered
HU-7	123	45°30′54″	82°27′06″	2.35	Silt
HU-8	72	45°31′30″	82°16′30″	1.91	Layered
HU-9	27	45°32′15″	82°02′54″	0.15	Sand/clay
HU-10	227	45°01′00″	82°01′00″	3.47	Silt
HU-11	38	45°55′10″	83°51′40″	0.27	Sand
SU-1	97	46°35′36″	84°49′30″	3.26	Silt
SU-2	121	46°45′48″	85°31′18″	0.92	Layered
SU-4	226	46°44′00″	86°32′48″	1.01	Silty sand/clay
	207			0.76	" "
	146			0.48	" "
SU-5	134	46°40′12″	86°28′12″	1.05	Layered
SU-6	362	47°00′00″	86°22′30″	3.57	Silt
SU-7	354	47°10′30″	86°16′45″	3.56	Silt
	328			3.21	Layered
SU-8	112	47°37′54″	85°49′48″	0.60	Silty sand

At station SU-7 two different sediment types were obtained. The first cast sampled silt in a depth of 354 m; the second and third casts obtained layered sediment from 327 and 329 m. Data from the two layered samples have been averaged in Table II, and are presented separately from the data from the single silt sample.

Station HU-10 in Lake Huron was at the greatest known depth in that lake (227 m; 124 fath), and SU-6 in Lake Superior was at 362 m (198 fath), a depth approaching that of the deepest sounding for Superior of 406 m (222 fath).

In Fig. 7 organic carbon has been plotted as a function of depth for Lakes Superior, Huron, and Michigan, and the points representing common sediment types connected as was done in Fig. 4. Bottom sediment types in Superior and Huron are essentially the same as those encountered in Lake Michigan. However, as noted in Table II, silty sand (and in one case sand) was underlain by

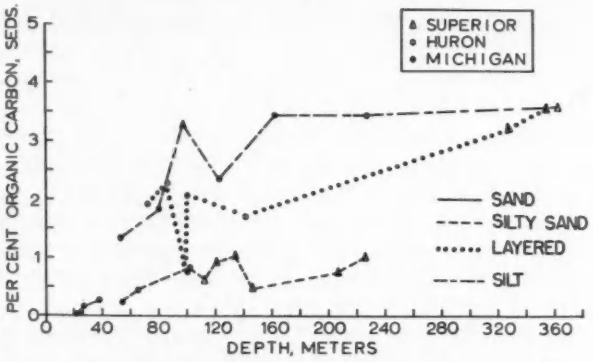

FIG. 7. Relation of organic carbon and depth in Lakes Michigan, Huron, and Superior, July 1966.

stiff plastic clay at a number of stations in Superior and Huron and was found in depths as great as 226 m (124 fath). This greatly exceeds the maximum depth for the sands found in Lake Michigan, where silty sand was not found deeper than 93 m (51 fath). The organic carbon content of these deeper sands, however, lies within the range of that found in the silty sand of Lake Michigan.

As was the case in Lake Michigan, the highest organic carbon values in Lakes Superior and Huron occurred in silt. Maximum values in all three lakes were about the same, around 3.5%, even though depths varied widely. In Lake Superior, organic carbon content of silt from 97 m (53 fath) and 362 m (198 fath) was 3.26% and 3.57%, respectively. Silt at 162 m (89 fath) in Lake Michigan contained 3.44% organic carbon, and at 227 m (124 fath) in Lake Huron, 3.47%. Although Nussman (1965) has reported values of 5.4% from two locations in Lake Superior at depths of 286 and 330 m (156 and 180 fath), per cent carbon was never found to exceed 4% during this study.

DISCUSSION

The analysis of the distribution of bottom types as presented here is from the point of view of the biologist attempting to discern interrelationships of sediment, organic matter, bottom topography, and water depth which may be of ecological significance. From the standpoint of the geologist, our breakdown of bottom types into only five basic categories is a gross oversimplication. However, these are categories which the biologist can easily learn to recognize in the field and, except for hard bottom, are quantitatively definable in terms of per cent sand, silt, and clay by laboratory analysis. In view of the often haphazard descriptions of bottom types encountered in reports dealing with benthos, these qualities of recognizability and quantitative verification justify their usage.

The distribution of the sediment types (excluding hard bottom) tends to be related to depth, although precise boundaries cannot be assigned to definite

depth ranges. Among all four types there are zones of overlap with respect to depth (cf. Fig. 5), but within these zones there is always a dominance of one sediment type. In Fig. 5, layered sediment predominates between 75 and 140 m (41 and 77 fath), silty sand occurs only once beyond 55 m (30 fath), and sand appears within the depth range of silty sand in only a single instance. Except for two small areas of layered sediment between Frankfort and Kewaunee, only silt is found at depths in excess of 140 m (77 fath). It is possible, then, on the basis of depth alone, to predict with some accuracy the kind of bottom sediment that will be encountered. The principal exceptions are the eastern side of the southern basin, where silt extends into relatively shallow water, and the areas of hard bottom.

The difference in sediment distribution between the eastern and western parts of the southern basin has been pointed out. Silt extends much closer to the eastern shore than to the western, where it is absent in depths less than about 90 m (49 fath). On the other hand, the silty sand belt is wider on the western side of the southern basin and thus extends farther into the lake than its east side counterpart. Further, the zones of hard bottom lying in the vicinity of Milwaukee–Racine and between Waukegan and Michigan City are peculiar to the west side. These patterns in sediment distribution within the southern basin strongly suggest conditions of net deposition on the east side and net nondeposition on the west.

Existing knowledge of circulation patterns in the lake is still sketchy, but Ayers et al. (1958), Bellaire (1964), and Verber (1966) have shown the existence of a large clockwise eddy which appears to occupy much of the eastern one-third of the southern basin. Such an eddy could constitute a mechanism for the deposition and accumulation of fine-grained sediments on the lake bottom.

In addition, all the major tributaries to the southern basin enter on the east side. These rivers are the Muskegon, entering at Muskegon, the Grand at Grand Haven, the Kalamazoo at Saugatuck, and the St. Joseph at Benton Harbor–St. Joseph. No tributaries of any consequence enter the southern basin of the lake from Benton Harbor around to Milwaukee. These four rivers are all large and at times carry heavy silt loads. It seems quite likely that they add significant quantities of material to the bottom sediments on their side of the lake and are thereby a contributing factor in the shoreward extension of silt on the east side of the basin. Cook and Powers (1964) showed that 3660 T of volatile suspended solids enter the lake annually from the St. Joseph River, and felt that it is reasonable to conclude that much of this settles to the lake bottom within a few miles of the harbor mouth. The rotational circulation in the eastern part of the southern basin, together with the large rivers which enter that side of the lake, may at least in part account for the proximity of silt to the eastern shore.

It seems probable that the distribution of layered sediment is also related to current activity. Although it is often found on steep slopes where slumping of surficial sediments might be invoked to explain the thinness of the upper

silt layer which rarely exceeds a depth of 1 inch, layered sediment also covers large areas of the floor of Lake Michigan in which the more regular topography does not suggest any such slippage of materials into deeper water. It appears, however, that horizontal currents of sufficient magnitude to transport sediment particle sizes as large as medium sand (0.25 mm on the Wentworth scale) occur at considerable depths in the lake. Such currents might be a primary factor preventing buildup of the upper silt component of layered sediment. Verber (1965) carried out studies in which recording current meters were suspended in the lake to depths of 180 m (98 fath) during the winter of 1962–63 and the summer of 1963. He shows average currents of 4.7 cm/sec at 90 m (49 fath), 3.2 cm/sec at 120 m (66 fath), and 2.7 cm/sec at 180 m (98 fath). Velocities in excess of 10 cm/sec were recorded at all of these depths at times. According to Sverdrup et al. (1946, p. 961), particle sizes as large as 0.3 mm (larger than medium sand) will be transported by a current with a velocity of about 2.5 cm/sec. Velocities of about 15 cm/sec and greater are required for erosion, but those of the magnitude measured by Verber are more than sufficient to prevent deposition of silt already in suspension. Since particulate organic material is of about the same density as water, it obviously would be subject to such transport as well.

The quantity of organic carbon found in the sediments is closely related to sediment type. In Fig. 5 it can be seen that silty sand, layered sediment, and silt are all present in the depth range 47–93 m (26–51 fath). However, within this depth range the organic carbon content for silty sand is 0.26–0.52%; for layered sediment, 1.25–1.69%; and for silt, 1.70–2.78%. Each sediment type exhibits its own range of organic carbon. Within sand, silty sand, and layered sediment there appears to be little relation between organic carbon content and depth when each sediment type is considered separately. Exceptions to this are the relatively high values for layered sediment in Lake Michigan at 216 m (118 fath) and in Lake Superior at 327 and 329 m (179 fath). It would appear, then, that whenever layered sediment is found at great depths the organic carbon content of the upper stratum is unusually large. In Lake Michigan there is a definite tendency for the organic carbon content of silt to increase with depth to bottom down to the 160–180 m depth range, with the highest values occurring beyond 160 m (88 fath). In the three lakes comparative study maximum organic carbon was likewise found in depths over 160 m, with no significant change being noted from that point into the greatest depths sampled.

The comparison of Lakes Michigan, Huron, and Superior with respect to organic carbon content of their sediments shows that maximum values are about the same in all three lakes. In those cases where it was possible to sample isolated basins, the maximum in all cases was between 3 and 4%. Such uniformity is in contrast to our findings that the lakes vary in their average amounts of particulate and dissolved organic matter and presumably also in amounts of organic production (Robertson and Powers, 1967). However, Gross (1967) found considerable areal variation in the organic carbon content

of surficial sediments of the northeast Pacific Ocean, in spite of the fact that Anderson (1964) noted little variation in primary photosynthetic productivity in the waters over the same region. Therefore, neither the present work not that of Gross indicate a relationship between quantity of organic matter contained in the surficial sediments and productivity of the overlying waters.

On the other hand, both Gross and Richards and Redfield (1954) have found clear-cut correlations between the quantity of organic matter in marine sediments and the dissolved oxygen content of the immediately overlying waters. In both cases maximum organic matter was found to correspond with minimum oxygen. These findings indicate that the organic matter contained in the surficial sediments is destroyed through oxidation, and thus progressive build-up of sedimentary organic matter cannot occur in the presence of oxygen-rich bottom waters. The deep waters of Lakes Michigan, Huron, and Superior are typically well oxygenated, with saturation values rarely falling below 80%. This could represent a controlling factor limiting the accumulation of organic matter in the surficial sediments of these lakes.

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Feeding of Some Great Lakes Fishes at Low Temperatures[1]

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ABSTRACT

Field studies on low temperature feeding in freshwater fishes were carried out as follows: (1) Fish Lake, near Picton, Ontario, January–March 1966 and 1967, 1200 fish netted from under the ice at a temperature of 4 C; (2) Little Cataraqui Creek near Collins Bay, April 1966, groups of fish (total, 544 individuals) netted at intervals of several days as the water temperature rose from 6.8 to 11 C; (3) Upper Jones Creek, near Mallorytown, two extensive collections (total, 1005 individuals) made between April 27 and 30, 1966 (at a water temperature of 8 C), and May 15 and 17 (15 C). The following findings resulted.

Some members of all six species in Fish Lake contained food but the percentage varied from 50% in *Umbra limi* down to 10% in *Fundulus diaphanus*. Hence feeding was erratic. Within species the smaller individuals invariably contained more food (0.005–0.02 g of alimentary contents per gram body weight) than larger fish (0.002–0.005). The same was true of smaller, as compared with larger, bodied species, with the exception of *F. diaphanus*.

After the thaw in Little Cataraqui Creek active feeding commenced at different temperatures in the various species: it was already under way at 6.5 C (when the study started) in *Ictalurus nebulosus* and *Pomoxis nigromaculatus*, but did not start in *Lepomis gibbosus* and *Ambloplites rupestris* until the water temperature reached 8.5 C. Prior to this the stomachs of species were shrunken, mucous-filled, and drawn far forward in the body cavity. Few *Perca flavescens*, active winter feeders, fed in April, immediately prior to spawning.

In most of the Jones Creek fishes there was a marked increase in weight of alimentary contents (gram per gram body weight) between 8 and 15 C. Exceptions were the larger *Semotilus atromaculatus*, which were than spawning, and *U. limi* and *Eucalia inconstans*, which were sampled before their peak daily feeding times. To the differences in weights of alimentary contents at 8 and 15 C must be added accelerated digestion rates at 15 C.

In all the waterways studied the fish consumed a much smaller range of prey items at low temperatures than at higher ones. This applied in midwinter, compared with summer, in Fish Lake, and in April and October, compared with summer, in Jones Creek. A few foods, e.g. chironomid larvae and Cladocera, were especially prominent in the diet at low temperatures, and species food specializations were suppressed. There was probably not, however, any increase in interspecific competition at temperatures approaching zero, because of the relatively low metabolic requirements of the fish.

INTRODUCTION

The present paper reviews food uptake at low temperatures in three faunas based on the occurrence, and weight, of food in the alimentary canal. Advantage is taken of slow stomach clearance rates below 10 C to develop a comparative picture of winter and early spring feeding.

The feeding of fishes at low temperatures has been largely neglected. Data are, however, a prerequisite to an understanding of the bioenergetic

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cycle in natural fish faunas, not to mention overwintering survival. Studies on Great Lakes fishes promise to be particularly interesting since the fauna is a combination of southern "warm water" and northern "cold-water" species, presumably characterized by different temperature optima. Fishermen have long recognised that whereas certain species (pike, lake trout) can readily be caught by hook and line through the ice, others can only rarely if at all (e.g. smallmouth bass) (Webster, 1954). Some Centrarchidae have been experimentally shown not to feed below 10 C (Marcus, 1932).

There is now ample experimental evidence to show that fish consume less food at low than high temperatures (Komarova, 1939; Hathaway, 1927; Marcus, 1932; Pentelow, 1939). Likewise, digestion times are notably decreased at low temperatures (Marcus, 1932; Molnar and Tolg, 1962; Molnar, 1968).

METHODS

The situations studied were as follows. (1) Feeding under the ice in Fish Lake near Picton, Ont., at a water temperature of 4 C between January and March 1966 and 1967. (2) Early spring feeding in Little Cataraqui Creek, near Collins Bay, Ont., as the water temperature rose from 6.5 to 11 C, April 1966. (3) Feeding during the late April–early May period of accelerated feeding with rapidly increasing water temperatures, in upper Jones Creek, near Mallorytown, Ont., Food uptakes at temperatures of 8 C (April 27–30, 1966) and 15 C (May 15–17) are contrasted.

In the first two studies the fish were collected by hoop net. The Jones Creek ones were taken by seine. That capture by hoop net over a 24-hr period at these temperatures gives comparable results with those taken by seine can be seen from the fact that the weights of stomach contents for the Fish Lake fish at 4 C are of the same magnitude as those obtained by the latter method in Jones Creek at 8 C (see later). Secondly, digestion at these temperatures is exceedingly slow. The hoop nets were left for 24-hr periods, at Fish Lake from 9.00 AM to 9.00 AM, and in Little Cataraqui Creek from 6.00 PM to 6.00 PM. The Jones Creek collections were made between 11.00 AM and 3.00 PM.

Fish Lake is a shallow eutrophic lake with a diameter of about 1 mile and a maximum depth of 10 ft. Winter ice-cover is 12–20 inches. Total dissolved solids are 218 ppm for July and 234 ppm for September (D. Sheppard, MS, based on analyses by the National Health and Welfare Laboratory, Kingston, Ont.). Six species compose the fish fauna (Tables I–III). Fish in this lake have previously been the subject of an extensive summer food study, based on 4500 stomachs (Keast, unpublished results).

Little Cataraqui Creek, a small stream draining an extensive marshy hinterland, enters Lake Ontario near its eastern limits. At the mouth, where the hoop nets were laid, it has a width of about 100 ft and a depth of 7–8 ft. A high flow rate during the spring thaw ensures constant water temperatures throughout the creek and the absence of diurnal variation. The upstream movement of fish, commencing in early April, is exploited by commercial fishermen and the fish analyzed here were chosen at random from their large daily catches.

The commonest five species are listed in Table IV. Post-spawning collections of *Perca flavescens* were kindly obtained by Dr D. Coble in the Bay of Quinte, Ont., after the commercial fishing ceased.

Upper Jones Creek is a series of channels and ponds ranging from a few to 15 ft across. It is a fairly productive stream. Total dissolved solids for the section from which the fish were taken were 156–160 ppm in July and 205–211 ppm in September (D. Sheppard, unpublished MS). Its fauna of nine species (Tables V–VII) has also been the subject of a summer food study, based on 8000 stomachs (Keast, 1966).

All fish were killed immediately, slit ventrally, and preserved in 10% formalin. Subsequently each was measured and weighed. When food was present in the alimentary canal it was carefully removed, superficially dried, and weighed.

The entire alimentary contents rather than those of the stomach alone were weighed, as the latter were usually too small to obtain a reasonable figure. The stomach material accounted a little less than half the total. The weight of alimentary contents per gram of body weight was then calculated for each individual, so that fish of different sizes would be comparable. Species with a marked size-range were divided into size-groups. Analyses of the kinds of food organisms are based on stomach contents only, the method followed being that described in Keast (1965, 1966). In this the results are expressed both numerically (numbers of organisms of each kind per 10 fish), and in terms of total per cent volume of each. Partially disarticulated organisms were counted as whole organisms.

In the Fish Lake and Jones Creek series the larger fish were divided into size-classes, arbitrarily chosen but with some attention to year-class limits, to bring out differences in food uptake associated with body size.

Species on which data are given belong to families as follows: Amiidae (*Amia calva*); Umbridae (*Umbra limi*); Esocidae (*Esox americanus vermiculatus*); Cyprinidae (*Chrosomus eos*, *Notemigonus crysoleucas*, *Notropis cornutus*, *Pimephales promelas*, *Semotilus atromaculatus*); Catostomidae (*Catostomus commersonii*); Ictaluridae (*Ictalurus nebulosus*); Cyprinodontidae (*Fundulus diaphanus*); Gasterosteidae (*Eucalia inconstans*); Serranidae (*Roccus americana*); Centrarchidae (*Ambloplites rupestris*, *Lepomis gibbosus*, *L. macrochirus*, *Micropterus dolomieui*, *M. salmoides*, *Pomoxis nigromaculatus*); and Percidae (*Etheostoma exile*, *Perca flavescens*).

WINTER FEEDING UNDER THE ICE, FISH LAKE

Occurrence of Food in Alimentary Canal

In all six species collected at Fish Lake some individuals contained food (Table I). The percentage of fish with empty alimentary canals was 35–52% in *U. limi*; 63–70% in *N. crysoleucas*; 57–76% in *I. nebulosus*; and 91, 77, and 78% in *F. diaphanus*, *E. inconstans*, and *E. exile*.

Molnar (1968) has recorded that, at 5 C, food remains in the stomachs of force-fed fish for 5–10 days. The species he studied included the European

TABLE I. Degrees of feeding by six fish species under the ice (water temperature 4 C), Fish Lake, Ont. Based on eight collections made between late January and mid-March 1966 and 1967. In *Notemigonus* (Cyprinidae) the first coil of the intestine is regarded as the stomach. Total number of fish: 1163.

Species	Total body length (*mm*)	No. of fish	Fish with no food in alimentary tract (%)	Individual with food in alimentary tract (%)		Avg weight alimentary contents per individual fish (including those without food) (*g*)	Weight alimentary contents per gram of fish	Degree of feeding indicated (based on columns 3 and 7)
				Stomach or stomach plus intestine	Intestine only			
Umbra limi	30–60	66	52	16	32	.01	.011	Moderate
	60–80	102	35	20	45	.023	.007	"
	80–124	94	40	10	50	.045	.005	"
Notemigonus crysoleucas	65–90	94	63	14	22	.025	.005	Little
	120–150	92	70	15	15	.008	.002	Little
Ictalurus nebulosus	40–55	38	57	17	26	.068	.018	Moderate
	70–120	94	74	18	7	.050	.006	Little
	125–150	68	76	16	8	.005	.0024	Very little
Fundulus diaphanus	35–80	190	91	1	8	.011	.002	Virtually nil
Eucalia inconstans	28–42	180	77	20	23	.007	.019	Little
Etheostoma exile	45–60	145	78	18	4	.008	.017	Little

perch (*Perca fluviatilus*), European trout-perch (*Lucioperca lucioperca*), and the largemouth bass (*M. salmoides*). Thus, separating individuals in which food remains occurred in the intestine only or not at all from those with food in the stomach, or in both stomach and intestine (Table I), should indicate those fish that had not fed for some days. Table I shows that at least half the individuals of all species had no food in the alimentary tract or food in the intestine only and that in *F. diaphanus* eight out of nine had no food. This confirms that at 4 C feeding is erratic.

In terms of weight of food in the alimentary canal the fish listed ranged between 0.02 and 0.002 g per gram body weight. Except for *F. diaphanus*, however, the real differences are not between species but between small and large fish. Thus, in the 30–60-mm group of *U. limi* the figure is 0.011 g, twice as high as in the 80–124-mm group (0.005 g). There is a similar difference between the small and large *N. crysoleucas* (0.005 and 0.002 g), whereas in *I. nebulosus* it is four times as high in the 40–55-mm as in the 125–150-mm group (0.018 g compared with 0.0024 g). The small-bodied species *E. exile* and *E. inconstans* (0.017 and 0.019 g, respectively) have relatively high values. These findings agree with what has been found with respect to difference in feeding rates of small and large fish at higher temperatures by Pearse (1924), Hathaway (1927), Arnoldi and Fortunatova (1937), Pearse and Achtenberg (1920), and Thompson (1941).

By considering columns 3 and 7 of Table I, the various fish species and size-classes can be broadly stratified in terms of amount of feeding at 4 C. *Umbra limi* and the smallest *I. nebulosus* were "moderate" feeders. At the other extreme, adult *F. diaphanus* and the larger *I. nebulosus* and *N. crysoleucas* consumed only negligible amounts of food.

Food Items Eaten

Cladocera and Ostracoda occurred in all fish, chironomid larvae and Copepoda in about two-thirds, and Amphipoda, Ephemeroptera nymphs, and molluscs in about half (Table II).

The six fish species differed from each other in that some consumed a wide range of foods and others only a narrow one. Thus, *U. limi* took 12 items; *I. nebulosus*, 10; *E. exile*, 8; *E. inconstans*, 6; *F. diaphanus*, 5; and *N. crysoleucas*, 3. In addition, some degree of food preference is suggested, though the volumes of food in most stomachs were too small for a proper assessment. Copepoda were prominent only in the 30–60-mm group of *U. limi*; algal remains were restricted to *N. crysoleucas*, and fish to the largest *U. limi* and *I. nebulosus*.

Winter feeding is compared with summer feeding in Table III, the summer data being based on the analysis of 4500 stomachs (Keast, unpublished results). Winter feeding was as diverse as summer feeding in *U. limi* and *I. nebulosus*, 9–10 major food types being eaten by each species. In *E. exile* and *E. inconstans* only two-thirds as many kinds of items were taken in winter as in summer,

TABLE II. Numbers of organisms of various groups (averages for 10 fish) found in stomachs of fish taken under the ice, Fish Lake, Ont., from late January to mid-March 1966 and 1967. Total body lengths (*mm*) and numbers of fish are given below the names of the species. Per cent volume refers to entire series. Total number of fish: 1163.

	Umbra limi						*Notemigonus crysoleucas*		*Ictalurus nebulosus*						*Fundulus diaphanus*		*Eucalia inconstans*		*Etheostoma exile*	
	30–60 66		60–80 102		81–124 94		65–150 186		40–55 38		70–120 94		125–150 68		65–100 190		28–42 180		45–50 145	
Food organisms	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.
Cladocera	6	25	6	3	5	2	40	55	70	70	200	35	140	30	8	60	42	65	2	2
Copepoda	12	45	3	1	1	<0.5			2	1	1						4	3	1	2
Ostracoda	5	30	6	3	3	3	1	5	6	26	20	20	4		1	5	1		1	3
Amphipoda			2	2	1	5							3	5	1	5	1	3	1	20
Isopoda			2	15	2	10														
Ephemeroptera nymphs					1	4					2	10	5	5			1	20	2	18
Zygoptera nymphs													1	5						
Anisoptera nymphs											1	10								
Coleoptera adults			4	25	1	20														
Trichoptera larvae					1	1														
Lepidoptera larvae			1	35																
Chironomid larvae					1				3	3	20	15	50	20	2	15	3	8	2	10
Gastropoda			3	15	2	5													1	35
Lamellibranchiata											1	5	6	10	2	15				
Annelida																			1	9
Fish — *Etheostoma*					1	50							1	30						
Plant remains								20												
Algae								20												

TABLE III. Diversity of winter and summer feeding compared, Fish Lake, Ont. Foods that reach 10% volumes of stomach contents are shown as "XX," lesser foods (3–9% volume) as "X." Size-classes of fish species are grouped. Summer data based on analysis of 4500 stomachs (Keast, unpublished data).

	Umbra limi		*Notemigonus crysoleucas*		*Ictalurus nebulosus*		*Fundulus diaphanus*		*Eucalia inconstans*		*Etheostoma exile*	
Food organisms	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Cladocera	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX		
Copepoda	XX					XX		XX	X	XX		XX
Ostracoda	XX	XX	X		XX	XX	X	XX		XX	X	
Amphipoda	X	XX			XX		X	XX	X		XX	XX
Isopoda	XX							XX				
Ephemeroptera nymphs	X	XX			X	XX			XX	XX	XX	XX
Plecoptera larvae												XX
Zygoptera nymphs					X	XX		XX				
Anisoptera nymphs					X							XX
Trichoptera larvae		XX				XX		XX				XX
Chironomid larvae					XX	XX	XX	XX	X	XX		XX
Chironomid pupae				XX				XX				
Diptera adults				XX				XX				
Lepidoptera larvae	X										X	
Hemiptera												XX
Coleoptera	XX	XX						XX		XX		
Gastropoda	X	XX		XX		XX		X			XX	XX
Lamellibranchiata					X		XX					
Annelida											XX	
Hydracarina		XX		XX		XX		XX		XX		
Fish	XX	XX			XX	XX						
Fish eggs										XX		
Filamentous algae			XX	XX								
Plant remains			XX	XX								
Total	10	9	4	7	9	10	5	13	5	8	6	9

in *N. crysoleucas* half, and in *F. diaphanus* one-third. *Fundulus diaphanus* is a particularly versatile feeder in Lake Opinicon in summer (Keast, 1965).

Differences in the diets of the six species in the two seasons were as follows.

Umbra limi.— Amphipods, Ephemeroptera nymphs, and gastropods were taken only in moderate amounts in winter, Trichoptera larvae and Hydracarina not at all. Copepods and isopods, prominent winter foods, were virtually absent from the summer diet.

Notemigonus crysoleucas.— Chironomid pupae, adult Diptera, Hydracarina, and gastropods, important in summer, were not found in the winter stomachs.

Ictalurus nebulosus.— Amphipods, prominent in winter, were virtually absent from the summer diet. Trichoptera larvae, Hydracarina, and gastropods were absent or unimportant in winter. Ephemeroptera and Zygoptera nymphs were utilized at both seasons but were more prominent in summer.

Fundulus diaphanus.— Too few stomachs contained food for conclusions to be drawn but the following items, prominent in summer, were absent from the winter stomachs: Isopoda, Zygoptera nymphs, Trichoptera larvae, chironomid pupae, Diptera adults, Coleoptera, Gastropoda, and Hydracarina.

Eucalia inconstans.— Ostracoda, Coleoptera, Hydracarina, and fish eggs were recorded only in summer.

Etheostoma exile.— Too few stomachs contained food for any conclusions to be drawn except that the winter diet was fairly diverse.

No data was obtained on the relative availabilities of the different invertebrates in Fish Lake in winter and summer. The absence of Trichoptera larvae and Hydracarina and rarity of gastropods in fish stomachs in winter suggest inaccessibility to fish at this time. In the case of *Fundulus*, which lacked many items obtained in winter by other species, functional inefficiency at low temperature could be involved.

BEGINNING OF ACTIVE SPRING FEEDING, LITTLE CATARAQUI CREEK

Occurrence of Food in the Alimentary Canal

Table IV lists the percentages of fish of the five most common species with empty alimentary canals.

Ictalurus nebulosus.— Feeding had already commenced at a water temperature of 6.5 C (April 14), and all fish contained moderate amounts of food in the stomach and intestine.

Pomoxis nigromaculatus.— Food was present in moderate amounts in 80% of fish on April 14. In several it was restricted to the stomach, and the intestine contained much thick white mucus or chyme, suggesting that feeding in these individuals had only just commenced. All fish collected after April 16 (temperature above 7 C) contained food.

Lepomis gibbosus.— At 6.5 C there was no trace of food in the alimentary canal, and stomachs were greatly contracted and drawn forward in the body

TABLE IV. Percentages of fish of the most common species with no food in alimentary canal on various days during approach of spring feeding, Little Cataraqui Creek, Ont., 1966. Water temperatures (C) are given below dates. Number of fish in each series is shown in parentheses. All fish in 150–260 mm total length range. Total number of fish: 534.

Species	Apr. 14 6.5		Apr. 16 6.5–7.0		Apr. 19 8.5		Apr. 22 9.5		Apr. 25 10.0		Apr. 30 10.5		May 2 11.5	
Ictalurus nebulosus	Nil	(22)	Nil	(16)	Nil	(15)	Nil	(15)	Nil	(15)	Nil	(15)		
Amblopltes rupestris	100	(20)	100	(20)	50	(20)	Nil	(15)	Nil	(15)	Nil	(15)		
Lepomis gibbosus	100	(24)	80	(20)	Nil	(20)	Nil	(15)	Nil	(15)	Nil	(15)		
Pomoxis nigromaculatus	20	(20)	13	(20)	Nil	(20)	Nil	(15)	Nil	(15)	Nil	(15)		
Perca flavescens	100	(20)	90	(20)	100	(20)	100	(15)	100	(15)	70	(15)	Nil	(12)

cavity. They and the intestines were plugged with mucus and it was obvious no feeding had taken place for a long time. Two fish netted on April 16 (temperature 6.5–7.0 C) had fresh food in the stomach; two had empty stomachs but food remains in the intestine; the remaining 16 were empty. Three days later (temperature 8.5 C), all fish were actively feeding, the stomachs and intestines containing moderate amounts of fresh food.

Ambloplites rupestris.— None of the fish taken at 6.5 and 7.0 C contained any food, the alimentary tract being full of mucus. In one fish some old fish vertebrae lay in the intestine. Three days later (8.5 C) half of the fish contained small amounts of food. On April 22 (temperature 9.5 C) all fish had moderately full stomachs.

Perca flavescens.— On April 16, although two sexually mature fish had a few items of food in the stomach, none of the others contained any trace of food. Active feeding commenced about April 30, immediately after ovulation. All individuals obtained on May 2 contained food. Feeding in *P. flavescens*, an active winter feeder, is known to be suspended prior to breeding (Pearse, 1919).

Other species.— Two large *M. salmoides* obtained on April 19 (temperature 8.5 C) had empty alimentary canals, although remains of a few fish vertebrae lay amongst the mucus in the intestine. One of three large *M. dolomieui* on this date contained 20 newly eaten chironomid larvae; the other two were empty. In all the *Micropterus* the tracts were somewhat shrunken and contained much mucous. Four *A. calva* and nine *Morone americana* collected on April 16 and 19 (temperatures 6.5–7.0 C and 8.5 C) were empty.

To obtain general data on the frequency with which commercially important species will accept baits at temperatures approaching zero enquiries were addressed to state fisheries authorities in New York and Michigan, where winter angling, through holes cut in the ice, is very popular. In Lake Chautagua and other lakes in northern New York *P. nigromaculatus* and *P. flavescens* are commonly caught by anglers through the ice, *M. salmoides* "bites fairly well," but *A. rupestris* takes bait rarely and "patchily," being obtained in some areas but not others (W. Bentley, personal communication). *Ictalurus nebulosus* is not commonly caught in winter but bites more readily at the beginning of spring when, as a result of the thaw, water starts moving under the ice. *Micropterus dolomieui* is never caught in winter. This agrees with the Cayuga Lake observations of Webster (1954): "inactive in winter, forming aggregations that lie in fissures in the bottom." In Michigan *P. nigromaculatus* and *P. flavescens* are readily obtained by anglers through the ice, *A. rupestris* occasionally, *M. salmoides* and *L. gibbosus* only rarely (Drs K. Linton and W. Gross, personal communication). Moffett and Hunt (1945) record that in Clear Lake, Michigan, the common species obtained by anglers are *P. nigromaculatus* and *M. salmoides*. Perch are "active winter feeders, eating much more than bluegills." No data are provided on *L. gibbosus* but *L. macrochirus* consumed "small amounts" of food in winter, with slight increases corresponding to prolonged periods of warm weather. In Wisconsin

P. nigromaculatus ceases feeding in autumn when the water temperature falls to 10 C and cannot be caught with either gillnets or lines between mid-October and mid-February (Pearse, 1919). They begin feeding in spring with the "first rise" in temperature.

That cold-blooded animals may adapt downwards when exposed to cold temperatures for long periods is known. Knauthe (1907), for example, has written that carp usually do not begin feeding in spring until the temperatures reach 6–8 C but after a hard winter, they will begin at 3–4 C. In a recent set of experiments with fish housed in indoor aquaria, the writer (unpublished data) has found that a wide range of fall-adapted fish species will take small amounts of food at 5 C, if slowly acclimated downwards over a month or more. Included in these are *L. gibbosus* and *I. nebulosus*.

Foods Taken by the Species

Pomoxis nigromaculatus fed almost entirely on predatory free-swimming chironomids of the genus *Procladius*, stomachs averaging 2400 per 10 fish. Amphipods were present in small numbers (60 per 10 stomachs) and a couple of fish contained single Ephemeroptera and Zygoptera nymphs. This simple diet contrasts with the diverse one of summer, when *Chaoborus* larvae, Diptera pupae, Cladocera, Ephemeroptera nymphs, and flying insects from the surface are taken in large numbers (Keast, 1968). Chironomid larvae do not usually rise above the 20% volume level in summer.

The *I. nebulosus* stomachs contained preponderantly chironomid larvae (500 individuals per 10 stomachs or 95% of the total volume). Dr A. L. Hamilton, Canada Department of Agriculture, Ottawa, identified these as exclusively tube-dwelling species. A high proportion of the larvae were full grown. Other items were occasional gastropods, isopods, amphipods, Trichoptera larvae, and leeches.

In *L. gibbosus* chironomid larvae accounted for 60% of the total volume of most series, and averaged 100–120 individuals per 10 stomachs. Other items were small numbers of bivalves, gastropods, amphipods, isopods, Trichoptera larvae, and Zygoptera and Anisoptera nymphs. The feeding of *L. gibbosus* was, accordingly, diverse. Volumes of chironomid larvae of up to 55% have been noted in *L. gibbosus* stomachs from Jones Creek during the summer (Keast, 1966).

Ambloplites rupestris contained only a few chironomid larvae, Amphipoda making up 60–75% of the stomach volumes. The numbers were small (about 110 per 10 stomachs). A few Trichoptera larvae, Hemiptera, Diptera pupae, and surface insects accounted for the remainder. *Ambloplites rupestris* of this size-group consume mostly crayfish in summer; smaller fish (75–115 mm long) take mostly Anisoptera and Ephemeroptera nymphs; only in 1st-year fish (length, 45-70 mm) do small items like Amphipoda and chironomid larvae predominate (Keast, 1965).

The two *P. flavescens* with food on April 16 contained 2 isopods, 20 chronomid larvae, and about 10 fish eggs. With the resumption of active feeding

in early May chironomid larvae and pupae were the prominent items in the stomachs, some isopods, amphipods, and copepods also being taken.

These food studies in April stress the great importance of chironomid larvae in the diet at this time. These larvae are very abundant in the substratum in April for there is then a "stacking up" of instars prior to emergence. Nevertheless, for so many species to be simultaneously feeding on chironomid larvae is unusual, especially since *A. rupestris* and *P. flavescens*, because of their thick jaw margins and large mouths (Keast and Webb, 1966), are ill-adapted for taking such small items.

ACCELERATED SPRING FEEDING WITH RISING TEMPERATURE, UPPER JONES CREEK, ONTARIO

Weight of Food in Alimentary Canal

The percentages of individuals in the various species and sizes with empty alimentary canals, and the average weights of alimentary contents per gram of fish for the April 27–30 (8 C) and May 15–17 (15 C) collections are compared in Table V.

All species contained food at the former temperature, except for *L. gibbosus* (not included on the Table), of which six specimens were collected. Of the total individuals of all species one-third had empty alimentary canals. Most *N. crysoleucas*, *N. cornutus*, *P. promelas*, and *E. inconstans*, contained food but only about one-third of *S. atromaculatus* did so. Two weeks later, at a water temperature of 15 C, all fish contained food except for the 125–180-mm group of *S. atromaculatus*. This was obviously because these fish were spawning, being concentrated in a few, small, weedy ponds.

The average weight of alimentary contents per gram of fish was at least three- to four-fold higher in several of the groups at the higher temperature, as in the 50–70-mm and 71–125-mm *S. atromaculatus*, 60–80-mm *N. cornutus*, all of *N. crysoleucus*, *P. promelas*, and *C. eos*, and the 40–60-mm and 65–90-mm *C. commersoni*. This order of difference corresponds fairly well with relative food uptake rates obtained in laboratory experiments at such temperatures (e.g., Hathaway, 1927). The weight per gram of fish in the largest *C. commersoni* was twice as great at the higher temperature as at the lower, but there was little difference in the 81–120-mm group of *N. cornutus*.

Three species or groups contained less food in the alimentary canal in mid-May than in late April: the largest group of *S. atromaculatus*, *E. inconstans*, and *U. limi*. In the last two species this could also have been due to the imminence of spawning, both being early breeders. More likely, however, sampling of the three species was carried out at the wrong time of day since, by 15 C, the aphasic feeding of low temperatures is replaced by cyclical feeding. Daily peaks of stomach fullness in the Jones Creek fishes for a water temperature of 13.5 C (September 24–26, 1966) have been derived by the method of Keast and Welsh (1968). Peak times for the various species were: *U. limi*, 3:00 PM and 12:30–1:15 AM; *C. eos*, 12:00 noon–3:00 PM; *P. promelas*, 12:00 noon and 9:30 PM; *S. atromaculatus*, 7:00 AM and 12:30–1:15 AM; *C. commersoni*,

TABLE V. Comparative food uptake at two temperatures, fish of upper Jones Creek, Ont., 1966.

Species	Total body length (*mm*)	Temp 8 C, Apr. 27–30 No. of fish: 544				Temp 15 C, May 15–17 No. of fish: 461			
		No. of fish	% empty	Avg weight (g) alim. contents per individual fish (including those without food)	Avg weight alim. contents per gram of fish	No. of fish	% empty	Avg weight (g) alim. contents per individual fish (including those without food)	Avg weight alim. contents per gram of fish
Umbra limi	45–70	34	25	.08	.03	30	0	.05	.016
Esox americanus vermiculatus	115–250	7	0	.323	.007	–	–	–	–
Chrosomus eos	45–60	86	42	.02	.02	68	0	.09	.09
Notemigonus crysoleucas	80–100	32	0	.10	.008	19	0	.53	.07
Notropis cornutus	60–80	22	20	.032	.009	20	0	.077	.028
	81–120	17	0	.30	.009	17	0	.25	.01
Pimephales promelas	48–58	34	0	.06	.029	44	0	.2	.09
	65–71	41	0	.04	.009	32	0	.12	.027
Semotilus atromaculatus	50–70	28	80	.038	.011	24	20	.118	.04
	71–125	23	50	.02	.008	28	66	.089	.03
	125–180	14	60	.215	.005	14	100	Nil	Nil
Catostomus commersonii	40–60	48	40	.01	.009	40	0	.047	.032
	65–90	33	50	.08	.009	40	0	.12	.025
	91–110	19	20	.112	.011	18	0	.215	.022
	120–220	17	20	.35	.009	24	0	.15	.018
Eucalia inconstans	35–50	89	10	.035	.032	43	0	.012	.012

9:00 AM and 6:30 PM; and *E. inconstans*, 6:30–9:30 PM. No data was obtained for *N. cornutus*. Hence, although the 11:00 AM–2:00 PM collecting time corresponded to the time of maximum stomach fullness for *C. eos* and *P. promelas*, it was a little late for the 7:00 AM peak of *S. atromaculatus* and the 9:00 AM one in *C. commersoni*. This was probably not of great importance as, at 15 C, the stomach clearance is still slow. On the other hand collections were completed ahead of the 3:00 PM peak of *U. limi*, and well ahead of the 6:30 PM one in *E. inconstans*. Accordingly, the small amounts of food in the stomachs of these species can be explained.

Foods Taken by the Species

Striking differences in the number of kinds of organisms eaten by several of the fish species between the end of April and mid-May will be seen from Tables VI and VII. On Table VIII a numerical summary is presented in which comparative data is included for late May, June, August, and October (from Keast, 1966). A greater diversity characterizes the mid-May samples, compared with the late April ones, in *C. eos* (7 items compared with 4), *N. cornutus* (9 and 3, 7 and 4), the smallest *S. atromaculatus* (10 and 6), and *E. inconstans* (10 and 7). There is little difference between the two collecting periods in *U. limi* (9 and 7), *N. crysoleucus* (3 and 4), *P. promelas* (4 and 5), and *C. commersoni* (8 and 9, 10 and 8).

The degree of diversity in mid-May is as great as that in late May, June, and August in *N. cornutus*, *S. atromaculatus*, *C. commersoni*, and *E. inconstans* (Table VIII). In *U. limi*, a greater diversity of organisms is consumed in June and August (13) than in mid-May (9), and also in *N. crysoleucus* (7–8 and 3). In *P. promelas*, which feeds largely on organic detritus in summer, slightly fewer food types are taken in summer (3) than in late April (5) and mid-May (4). By late October (temperature 8–10 C) there is a marked drop-off in the diversity of foods eaten so that for virtually all species the numbers of kinds of food organisms consumed are about the same as for May.

The major food organisms absent, or virtually absent, from the fish stomachs in April were: Coleoptera, Amphipoda, Hemiptera, Ephemeroptera nymphs, Hydracarina, and molluscs (Table VIII). This presumably reflects their relative absence, or inaccessibility, in the environment.

The prominence of Diptera larvae or pupae in the diet immediately subsequent to the thaw was noteworthy, these being predominant in the diet of six of the eight species in April (Table VI): *C. eos*, *N. crysoleucus*, the smaller *N. cornutus*, *P. promelas*, *C. commersoni*, and *E. inconstans*. Simulid larvae, accounting for 65–75% of stomach volumes in some species were the most prominent group of Diptera. These organisms are particularly numerous in Ontario streams in April, immediately prior to emergence of the adults. These larvae and pupae reached 316 per 10 stomachs in the largest *C. commersoni*, and 74 per 10 stomachs in the small *E. inconstans*.

By May 15 there was a markedly reduced emphasis on Diptera larvae and pupae, and these now predominated in the diets of only the three larger

sizes of *C. commersoni* and *E. inconstans* (Table VII). Filamentous algae, which proliferate as the temperature rises, accounted for most of the stomach contents of *N. crysoleucus* and *N. cornutus*, as did organic detritus in *C. eos* and *P. promelas*. These are the typical summer foods of these species (Keast, 1966). Adult and larval Coleoptera and Hydracarina, prominent by May 15, are common in the diets of several species throughout summer (Keast, 1966).

By mid-May it is obvious that the summer food specializations of the various Jones Creek species, detailed in Keast (1966), were already established. The major overlaps in kinds of food between species were thus reduced before the onset of accelerated feeding that came with higher temperatures.

CONCLUSIONS

The following provisional conclusions emerge from the present work:

(1) Under the ice in mid-winter, at a temperature of 4 C all the fish species of Fish Lake, Ont., took some food. The amount of food in the alimentary canals of the various species varied from insignificant amounts (0.002 g per gram body weight) in the larger-bodied members of *I. nebulosus* and in the small-bodied species *F. diaphanus* to 0.02 g in the smallest group of *I. nebulosus* and in *E. inconstans*. Less than half this amount was in the stomach. The erratic nature of winter feeding, and species differences in frequency of feeding, can be seen from the percentages of individuals with empty alimentary canals, 35–40 % in *U. limi*, and 91% in *F. diaphanus*.

(2) The amount of food taken was obviously inadequate to maintain body weight in several of the species. *Fundulus diaphanus*, *E. exile*, and the smaller *I. nebulosus* all had very thin bodies in February and March. Both small and large *U. limi* were, however, in excellent condition. That "condition factor," the ratio of body weight to body length, is commonly low in winter is widely recognized (Cooper and Benson, 1951; Cooper, 1953). This suggests that conditions under the ice in Fish Lake were not exceptional.

(3) After the thaw in April, in all large *L. gibbosus* and *A. rupestris*, and a couple of *M. salmoides* and *M. dolomieu*, netted at the entrance to Little Cataraqui Creek at water temperatures of 6.5–8 C, the stomachs were shrunken, they contained thick white mucus and were drawn far forward in the body cavity. It was obvious that none of these fish had fed for many weeks. The commencement of feeding in the first two species at 8.5 C was very clear-cut.

(4) Most of the larger game fishes, including certain centrarchids, can be taken by hook and line through the ice in winter. The evidence from New York and Michigan, however, is that *A. rupestris* bites only rarely and *L. gibbosus* scarcely ever. *Micropterus dolomieu* hibernates in winter in rock fissures at the bottom of lakes (Webster, 1954).

(5) The present work does not show whether the low level of winter feeding in fish is due to the paucity of available food organisms or to the inability of fish, because of their reduced metabolism at low temperatures, to forage sufficiently widely to obtain a reasonable ration. Probably both are involved. Some insight is coming from the writer's current work in aquaria. It is being found that most species, including *L. gibbosus*, *F. diaphanus*, and *I. nebulosus*, will take food at 5 C if acclimated to this temperature over a prolonged period. *Umbra limi*, however, adapts to this temperature very rapidly. When small, live commercial redworms (Oligochaeta) are presented in excess at 5 C a mean daily uptake four to eight times that calculated for Fish Lake at 4 C is consumed by *U. limi*, and 5–10 times the amount for Fish Lake by *F. diaphanus*. The two sets of figures are not truly comparative in that an excess of food in a confined space is obviously a very different situation to that pertaining in the wild. The differences may also be exaggerated by the bulkiness and high water content of the oligochaetes fed in the aquaria. After a time on this feeding regime the aquaria fish become heavier but do not increase in length.

(6) There was little difference in stomach contant weights between 5 and 8 C (compare the Fish Lake series (Table I) with those for Jones Creek for April 27–30 (Table V)) but a marked increase in most species between 8 and 15 C. Added to this three- to four-fold increase in weights in most species is the faster digestion rate at 15 C.

TABLE VI. Numbers and volumes of organisms of various groups found in stomachs of Upper Jones Creek fishes at a water temperature of 8 C, April 27–30, 1966. Total body lengths (mm) and numbers of fish are given below the names of the species. Number of organisms is average for 10 fish. Per cent volume, given for 1% or higher, refers to entire series. Number of fish: 537.

	Umbra limi		*Chrosomus eos*		*Notemigonus crysoleucas*		*Notropis cornutus*				*Pimephales promelas*		*Semotilus atromaculatus*						*Catostomus commersonii*								*Eucalia inconstans*	
	45–70		45–60		80–100		60–80		81–120		50–70		50–70		71–125		121–165		40–60		65–90		91–110		120–220		35–50	
	34		86		32		22		17		75		28		23		14		48		33		19		17		89	
Food organisms	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.
Cladocera											1								12	20	42	12	29	1	4		1	
Copepoda	4	3																	6	10	59	20	127	7	42	1		
Ostracoda	14	10																	4	5	73	18						
Amphipoda	7	35																										
Isopoda													1	10														
Ephemeroptera nymphs																	4	4							2	1	1	
Lepidoptera larvae															1	35									2	20		
Zygoptera nymphs	4	10			3	8			21	60			2	15	1		1	1										
Anisoptera nymphs																												
Trichoptera larvae	4	15			10	15					1	1	3	30	7	30	5	25	1	3	2	5	4	2	2	1	1	1
Chironomid larvae	8	5	1		6	7	14	6	20	4	5	8	1	5	3		1		5	18	55	20	107	25	160	10	20	10
Chironomid pupae			2	10			24	28	60	16	6	6	4	30	1				3	15	5	3	44	50	4	7	10	15
Diptera adults			1	5																								
Simulid larvae			20	75	47	70	90	65	110	20	65	75			47	5			3	15	13	15	4	4	268	35	62	50
Simulid pupae			3	10																			15	10	48	25	12	20
Hemiptera															1		2	5										
Coleoptera	4	20																										
Gastropoda																												
Pelecypoda													1	5														
Hydracarina																											3	1
Hirudinea															1	25												
Porifera																			2	8	21	2	3					
Fish — *Eucalia*																	3	60										
Fish eggs																	89	5							2	1	2	2
Filamentous algae												7								5		5						
Plant remains																5												

(mm) and numbers of fish are given below the names of the species. Number of organisms is the average for 10 fish. Per cent volume, given for 1% or higher, refers to entire series. Number of fish: 461.

Filamentous algae										7							5		5							
Plant remains													5													

(mm) and numbers of fish are given below the names of the species. Number of organisms is the average for 10 fish. Per cent volume, given for 1% or higher, refers to entire series. Number of fish: 461.

	Umbra limi		*Chrosomus eos*		*Notemigonus crysoleucas*		*Notropis cornutus*				*Pimephales promelas*		*Semotilus atromaculatus*						*Catostomus commersoni i*								*Eucalia inconstans*	
	45–70 30		45–60 68		80–100 19		60–80 20		81–120 17		50–70 76		50–70 24		71–125 28		126–165 14		40–60 40		65–90 40		91–110 18		120–220 24		35–50 43	
Food organisms	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.	No.	% vol.
Cladocera			2	1															32	9	45	8	8		90		27	4
Copepoda	28	5																	29	8	78	15	68	2	90	5	6	1
Ostracoda	31	8	1	1															6	2	19	1					5	1
Amphipoda																									2			
Isopoda													1	1														
Ephemeroptera nymphs													1		3	15									6	2		
Plecoptera larvae							1	2			1	1													3	5		
Zygoptera nymphs													1	15			Empty											
Anisoptera nymphs							3	25																				
Trichoptera larvae	3	12					2	30	4	3			3	25	6	25											1	1
Chironomid larvae	27	15	2	1	20	2	4	1	29	4	2	2	2		3				50	10	44	40	65	5	249	35	21	15
Chironomid pupae							19	10	14	10	1		6	5					1	3	13	2	16	4	69	17	1	3
Simulid larvae			1	1			11	8	14	2	6	6	18	10	59	20			1	1	2	2	280	70	308	25	4	55
Simulid pupae	5	1					4	5	1	1			6	5									16	5	18	3	3	3
Hymenoptera							1	3					2	2														
Hemiptera													1	3														
Coleoptera	5	30					3	15	1	5	1		6	20							2	2	5	1	8	3		
Gastropoda	3	2													5	10									3	1		
Pelecypoda	5	2																							10	2		
Hydracarina	32	25	3	1	20	2	2						1						5	2	13	2	10	1	5			
Araneida															1	15												
Hirudinea													1	12	1	15											10	6
Turbellaria			1	1																								
Porifera																			55	60	45	18	19	2				
Fish eggs																											7	10
Filamentous algae						96				75																		
Organic detritus				95								90								5		10		10				

TABLE VIII. Numbers of kinds of food organisms (accounting for 1% or more of the total volume) found in stomachs of eight species of Jones Creek fishes at various times of year (data for last four columns extracted from Keast (1966), based on a 4-year food study between 1962 and 1965). The number of fish in each sample is shown in parentheses. *Notemigonus crysoleucus* and the larger *N. cornutus* vacated the sampling area before October.

Species	Total length (*mm*)	Apr. 27–30 8 C	May 13–15 15 C	May 18–26 16–19 C	June 18–26 18–22 C	Aug. 18–26 20–25 C	Oct. 18–26 8–10 C
Umbra limi	45–70	7 (34)	9 (30)	12 (38)	13 (39)	13 (40)	8 (35)
Chrosomus eos	45–60	4 (86)	7 (68)	5 (28)	5 (22)	8 (29)	2 (25)
Notemigonus crysoleucus	80–100	4 (32)	3 (19)	7 (41)	8 (38)	7 (35)	Absent (Sept. 18–26, 4(36))
Notropis cornutus	60–80	3 (22)	9 (20)	6 (29)	8 (30)	7 (39)	5 (22)
	81–120	4 (17)	7 (17)	7 (34)	7 (28)	5 (18)	Absent (Sept. 18–26, 2 (19))
Pimephales promelas	50–70	5 (75)	4 (76)	2 (27)	3 (26)	3 (19)	3 (18)
Semotilus atromaculatus	50–70	6 (28)	10 (24)	10 (32)	8 (39)	13 (45)	9 (28)
	121–165	6 (14)	Not feeding (14)	4 (28)	3 (20)	5 (29)	5 (21)
Catostomus commersonii	40–60	9 (47)	8 (40)	10 (32)	7 (36)	6 (30)	7 (18)
	120–220	8 (17)	10 (24)	12 (26)	7 (28)	12 (32)	10 (12)
Eucalia inconstans	35–50	7 (89)	10 (43)	9 (36)	9 (54)	8 (47)	7 (39)

(7) The Fish Lake, Little Cataraqui Creek, and Jones Creek studies all indicate that winter feeding is directed at a relatively few kinds of food items. There is support for this in the studies of other authors. Thus *P. flavescens* in Oneida Lake, New York, feeds solely on mayfly nymphs in February (Adams and Hankinson, 1928). Ostracoda predominate in the diet of *L. macrochirus* in Cedar Lake, Michigan in mid-winter (Moffett and Hunt, 1945). This food was prominent in the winter diet in Fish Lake, as it is in the diet of fish in early spring in both Lake Opinicon and Jones Creek. Interestingly, Ostracoda are largely rejected by fish in late summer, notwithstanding the great abundance of these organisms then (Keast, 1965, 1966).

(8) In Jones Creek there is a marked increase in diversity of feeding from April to May, with a further increase in some species between May and August, then a marked falling off in October down to about the April levels.

(9) The competitive interrelationships between cohabiting species are obviously different at low temperatures in winter from these in summer. Food consumption is greatly reduced. There is marked food overlap. Species may take foods that they do not eat commonly at other times, as Diptera larvae are taken by *A. rupestris* and *P. flavescens* in Catarqui Creek and by *P. promelas* and *C. eos* in Jones Creek. This study does not show whether or not there is any increase in interspecific competition in winter. However, the low caloric requirements of most species, and their capacity to fast for long periods, would suggest not.

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An Estimate of Ocean Mortality of Bristol Bay Sockeye Salmon Three Years at Sea[1,2]

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ABSTRACT

The method of population estimation based on change in composition of a dichotomous population was applied to Bristol Bay sockeye salmon to estimate the mortality rate of 3-ocean (3 years at sea) fish during their final year in the ocean. A difference in freshwater age composition between 1-ocean immatures in 1964 and 2-ocean immatures in 1965 was evident in samples taken in the high seas. This difference was apparently due to a greater tendency of 2-freshwater fish than 1-freshwater fish to mature after 2 years at sea. The known quantities of these two freshwater categories in the mature 2-ocean run of 1965 enabled an estimate of the population of 2-ocean immatures at sea in 1965. From this estimate and the quantity of 3-ocean matures in the 1966 run, the annual mortality rate was estimated to be .42.

INTRODUCTION

This paper presents an application of the method of population estimation based on change in composition due to selective removal to Bristol Bay sockeye salmon (*Oncorhynchus nerka*) to estimate the natural mortality rate of 3-ocean (3 years at sea) fish during their penultimate year. This method has been widely used to estimate populations of game birds and mammals (Dasmann, 1952). Murphy (1952) used change in the sex ratio of a coho salmon (*O. kisutch*) population to estimate the total mortality rate during the last year of life. Lander (1962) showed how fishing mortality rates and, in specific situations natural mortality rates, could be estimated from the change in composition of a dichotomous population.

Most of the recently published methods of estimating ocean mortality rates of Pacific salmons (Ricker, 1962; Parker, 1962; and Fredin, 1965) rely on inshore observations of the population of outgoing smolts and adult returns. One of several methods developed by Fredin was based on indices of abundance of immature Bristol Bay sockeye at sea.

METHOD

The size of a closed population can be estimated from a change in composition when the population is readily separable into two classes — call these X and Y — and when there is selective removal or exploitation of one

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of these classes, say class X, to the extent that the proportion of the total population in this one class is considerably reduced. The principle, which can be extended to cases involving any number of classes, is the solving of a system of simultaneous equations (two equations when there are two classes). The quantities that must be known or estimated in order to estimate the size of the population before removal are the proportion of the population in class X before removal, the proportion in class X after removal, and the numbers of X and Y taken from the population by the source of removal or exploitation.

Chapman (1954) presents the maximum likelihood estimator for the initial population size and the asymptotic variance of the estimate for the case in which there is no source of removal other than that for which a measure is obtainable. Chapman's basic formulations are applicable with slight modifications to suit this specific situation with the assumption that removal occurs instantaneously.

APPLICATION

Most of the Bristol Bay sockeye salmon migrate to sea in either their 2nd or 3rd year of life, after spending one or two winters, respectively, in fresh water. Most of these mature after 2 or 3 years at sea. Thus about 99% of the mature Bristol Bay run usually consists of four age-groups (Fredin, 1965), designated in this paper as 1.2, 1.3, 2.2, and 2.3[4] The first numeral is the number of winters spent in fresh water, and the second numeral is the number of winters spent at sea. These four age-groups in their penultimate year are classified 1.1, 1.2, 2.1, and 2.2, respectively, and fish which mature after spending 3 years in the ocean are designated 1.1 and 2.1 in their antepenultimate year. Sockeye salmon spending 2 and 3 winters at sea are designated as 2-ocean and 3-ocean fish, respectively; similarly, sockeye salmon that spend 1 and 2 winters in the lake are referred to as 1-freshwater and 2-freshwater fish, respectively.

The X class in this application consists of 2-freshwater (2.) sockeye and the Y class consists of 1-freshwater (1.) sockeye. Selective removal of these two classes from the ocean feeding grounds after 2 years at sea apparently occurs as a result of a greater tendency of 2-freshwater Bristol Bay sockeye than of 1-freshwater sockeye to mature after 2 rather than 3 years at sea. We can expect, then, a change in freshwater age composition within the 2-ocean group at sea at the time the maturing 2-ocean fish migrate inshore. In year i, the 1-ocean group will contain two freshwater age-groups, 1.1 and 2.1. The following year $(i+1)$, within the same group (now 1.2 and 2.2) a greater proportion of the 2.2's are expected to migrate shoreward than 1.2's. Thus in high seas samples drawn during the summer of year i, the ratio $2.1:(2.1+1.1)$ will be greater than the ratio $2.2:(2.2+1.2)$ in samples drawn after the exodus of matures in year $i+1$.

[4]Following the European system of age designation.

In 1964 and 1965 the Fisheries Research Institute collected with commercial purse-seine gear and tagged large numbers of immature sockeye in the North Pacific Ocean immediately south of Adak Island. These samples were taken in the period June 15–August 15. By June 15 practically all of the 2-ocean sockeye found south of Adak are immature since most of the mature 2-ocean fish have left for Bristol Bay. Data on inshore tag recoveries (Table I) show that the immatures in the Adak area during the tagging period

TABLE I. Inshore recoveries of sockeye salmon tagged as immatures by the Fisheries Research Institute south of Adak Island in 1964 and 1965.

Year of tagging	Ocean age	No. tagged	1965 inshore recoveries		1966 inshore recoveries	
			Bristol Bay	Elsewhere	Bristol Bay	Elsewhere
1964	1	3677	26[a]	0	9	1[b]
1964	2	312	5	0	0	0
1965	1	1512	–	–	1	0
1965	2	1006	–	–	21[c]	0

[a]Including one from the south side of the Alaska Peninsula (probably destined for Bristol Bay).
[b]Cook Inlet, Alaska.
[c]Including two from the south side of the Alaska Peninsula (probably destined for Bristol Bay).

in both 1964 and 1965 were of almost pure Bristol Bay stock. Age analysis of the 1964 and 1965 high-seas samples, known quantities of 1.2 and 2.2 mature sockeye in the 1965 Bristol Bay run and 3-ocean matures in the 1966 run, and an estimate of the catch of immature Bristol Bay sockeye (2-ocean fish) by the Japanese high-seas fishery in the summer of 1965 provide the basis for estimating the population of 2-ocean immatures in the summer of 1965. For clarification the maturing segment of the 2-ocean group separates from the immature segment at some unknown time prior to the beginning of the sampling period at Adak. Therefore samples from two consecutive summers had to be used rather than samples from the same calendar year before and after exodus of the matures.

The Japanese fishery takes mature Bristol Bay sockeye salmon approximately 2–6 weeks before the run arrives inshore. The runs of mature 2-ocean fish to Bristol Bay in 1965 and 3-ocean fish in 1966 in this analysis include the estimated catches by the Japanese from these two groups. The criteria for estimating both the mature and immature catches of Bristol Bay sockeye salmon by the Japanese are stated by Ossiander (1965).

An assumption required for this analysis is that the rate of natural mortality depends only on ocean age and is therefore independent of sex, maturity, or freshwater age. Since the major determinant of the size of sockeye salmon

at sea is their ocean age, this assumption is reasonable. Thus all 1-ocean sockeye are assumed to be exposed to the same average rate of mortality, and all 2-ocean immatures are likewise assumed to be subject to a common average rate, which may differ from that experienced by 1-ocean fish.

On the assumption that the departure of mature sockeye from the ocean feeding grounds to Bristol Bay takes place instantaneously at the end of June, the following definitions apply:

$n_{x.i}$ = number of immature 2.1 sockeye in Adak sample in year i

$n_{y.i}$ = number of immature 1.1 sockeye in Adak sample in year i

$n_{z.i} = n_{x.i} + n_{y.i}$

$n_{x.i+1}$ = number of immature 2.2 sockeye in Adak sample in year $i+1$

$n_{y.i+1}$ = number of immature 1.2 sockeye in Adak sample in year $i+1$

$n_{z.i+1} = n_{x.i+1} + n_{y.i+1}$

$N_{x.i}$ = millions of immature 2.1 sockeye at sea on July 1 of year i

$N_{y.i}$ = millions of immature 1.1 sockeye at sea on July 1 of year i

$N_{z.i} = N_{x.i} + N_{y.i}$

S_1 = annual survival rate of 1-ocean sockeye on July 1 of year i to 2-ocean sockeye on June 30 of year $i+1$

$N_{x.i}S_1$ = millions of 2.2 sockeye (mature and immature combined) at sea on June 30 of year $i+1$

$N_{y.i}S_1$ = millions of 1.2 sockeye (mature and immature combined) at sea on June 30 of year $i+1$

$N_{z.i}S_1 = N_{x.i}S_1 + N_{y.i}S_1$

$N_{x.i+1}$ = millions of immature 2.2 sockeye at sea on July 1 of year $i+1$

$N_{y.i+1}$ = millions of immature 1.2 sockeye at sea on July 1 of year $i+1$

$N_{z.i+1} = N_{x.i+1} + N_{y.i+1}$

$R_{x.i+1}$ = millions of mature 2.2 sockeye in run of year $i+1$

$R_{y.i+1}$ = millions of mature 1.2 sockeye in run of year $i+1$

$R_{z.i+1} = R_{x.i+1} + R_{y.i+1}$

$J_{z.i+1}$ = millions of immature 1.2 and 2.2 Bristol Bay sockeye in Japanese catch of year $i+1$

S_2 = annual survival rate of 2-ocean immature sockeye on July 1 of year $i+1$ to 3-ocean matures on June 30 of year $i+2$

$R_{z.i+2}$ = millions of mature 3-ocean sockeye (1.3 and 2.3) in run of year $i+2$

Now, let p_1 be the proportion of 2-freshwater sockeye in the 2-ocean population immediately before the mature 2-ocean fish leave for Bristol Bay. Let p_2 be this proportion after the 2-ocean matures have left. If 2-freshwater fish have a greater tendency to mature after 2 years in the ocean than 1-freshwater fish, then p_2 will be less than p_1. Then, from the previous definitions,

$$p_1 = \frac{N_{x.i}S_1}{N_{z.i}S_1} = \frac{N_{x.i}}{N_{z.i}}; \qquad \ldots\ldots(1)$$

$$p_2 = \frac{N_{x.i+1}}{N_{z.i+1}} = \frac{N_{x.i}S_1 - R_{x.i+1}}{N_{z.i}S_1 - R_{z.i+1}} \qquad \ldots\ldots(2).$$

The 1964 Adak sample provided an estimate of p_1, and since the Japanese fishery on immature 2-ocean fish was probably nonselective as to freshwater age and furthermore was relatively light in 1965, the 1965 Adak sample provided an estimate of p_2.

$$\hat{p}_1 = \frac{n_{x.i}}{n_{z.i}} \; ; \; \hat{p}_2 = \frac{n_{x.i+1}}{n_{z.i+1}} .$$

When these estimates are substituted for p_1 and p_2 in equations 1 and 2, the resulting equations can be solved simultaneously to give an expression for the population of 2-ocean sockeye, both mature and immature, on June 30 of year $i+1$. This is the well-known dichotomy population estimate.

$$\widehat{N_{z.i}S_1} = \frac{R_{x.i+1} - \hat{p}_2 R_{z.i+1}}{\hat{p}_1 - \hat{p}_2} \qquad \ldots\ldots(3).$$

From this must be subtracted the mature 2-ocean run and the Japanese immature catch in year $i+1$, for the number of 2-ocean immature sockeye remaining at sea in July of year $i+1$ to be estimated:[5]

$$\hat{N}_{z.i+1} = \widehat{N_{z.i}S_1} - R_{z.i+1} - J_{z.i+1} \qquad \ldots\ldots(4).$$

Finally, S_2 is estimated.

$$\hat{S}_2 = \frac{R_{z.i+2}}{\hat{N}_{z.i+1}} \qquad \ldots\ldots(5).$$

For the specific application, the following values were required (the statistics for the Bristol Bay run are from Table II):

$n_{x.i}$ = 3975	$n_{y.i}$ = 431	$\hat{p}_1$ = .902
$n_{x.i+1}$ = 1100	$n_{y.i+1}$ = 373	$\hat{p}_2$ = .747
$R_{x.i+1}$ = 54.409 million		
$R_{y.i+1}$ = 1.388 "		
$J_{z.i+1}$ = .561 "		
$R_{z.i+2}$ = 15.007 "		

When these values are used in equations 3, 4, and 5,

$$\widehat{N_{z.i}S_1} = 82.122 \text{ million,}$$
$$\hat{N}_{z.i+1} = 25.764 \text{ million, and}$$
$$\hat{S}_2 = 0.582$$

For the time that S_2 applies (assuming the Japanese immature catch was taken instantaneously on July 1) the only deaths that occurred in the

[5]The Japanese immature catch is actually taken in July and August, but it is of no great consequence to assume it is taken instantaneously on July 1 as equation 4 specifies.

TABLE II. Statistics on the 1965 and 1966 Bristol Bay sockeye runs which were used to estimate the ocean mortality of 3-ocean fish in their penultimate year.

	Inshore catch and escapement (*in millions*)	Estimated high-seas catch (*in millions*)	Total (*in millions*)
1965 (mature 2.2)[a]	49.371	5.038	54.409
1965 (mature 1.2)	1.294	.094	1.388
1965 (immature 1.2 + 2.2)	–	.561	.561
1966 (mature 3-ocean)	13.471	1.536	15.007

[a]*Sources*: 1965 inshore run: Pennoyer and Seibel (1966); 1966 inshore run: personal communication, Mr Frank Ossiander, U.S. Bureau of Commercial Fisheries, Montlake Laboratory, Seattle, Wash.; 1965 high-seas catches: compiled by Mr R. A. Fredin, U.S. Bureau of Commercial Fisheries, Montlake Laboratory, Seattle, Wash., from age composition data sent to him by the Japanese Fishery Agency, and catch statistics given in the International North Pacific Fisheries Commission Statistical Yearbook, 1965; 1966 high seas catch: preliminary figure compiled by Mr R. A. Fredin.

population were natural deaths. Thus an estimate of the average monthly instantaneous rate of natural mortality (M) can be made for the final 12 months of life.

$$\hat{M} = \frac{-\log_e \hat{S}_2}{12} = \frac{-\log_e(.582)}{12} = .045 \qquad \ldots\ldots(6).$$

Statistical confidence limits for S_2 and M can be approximated. Assume that the only quantity to be estimated in the right-hand side of equation 4 is $N_{x.i}S_1$; then

$$\text{var}\,(\hat{N}_{x.i+1}) = \text{var}\,(\widehat{N_{x.i}S_1}).$$

According to Chapman's (1954) expression for the variance of the population estimate,

$$\text{var}\,(\widehat{N_{x.i}S_1}) =$$

$$\left(\frac{(\widehat{N_{x.i}S_1}\widehat{N_{y.i}S_1}\,\hat{p}_1^2)}{n_{x.i}} + \frac{(\widehat{N_{x.i}S_1} - R_{x.i+1})\,(\widehat{N_{y.i}S_1} - R_{y.i+1})\,\hat{p}_2^2}{n_{x.i+1}}\right) \Big/ (\hat{p}_2 - \hat{p}_1)^2 \qquad \ldots\ldots(7).$$

Finally, the variance for $\hat{S}_2$ can be approximated:

$$\text{var}\,(\hat{S}_2) = \frac{R_{x.i+2}^2}{\hat{N}_{x.i+1}^4}\left[\text{var}\,(\widehat{N_{x.i}S_1})\right] \qquad \ldots\ldots(8).$$

When values from the 1964-66 period are used in equations 7 and 8, var $(\hat{S}_2)$ = .00341. With the assumption that the estimate of S_2 is approximately normally distributed, 95% confidence limits for S_2 are .582 ± .117.

These limits can be transformed directly to give lower and upper confidence limits for M, which are .028 and .064.

DISCUSSION

In the present application the high-seas samples from which p_1 and p_2 were estimated were reasonably large, and there was a substantial difference between the two estimated proportions. Therefore, statistically efficient estimates of S_2 and M were obtained. The estimates of p_1 and p_2 were apparently not seriously in error from any major quantity of non-Bristol Bay sockeye in the samples. For all other pairs of years since 1956, when sampling at Adak was first undertaken, either the samples were too small in year i or $i+1$ to provide precise estimates, or tag returns indicated greater relative abundance of non-Bristol Bay immatures in the Adak area than in 1964 and 1965 (Hartt, 1962, 1966). It should be pointed out that equation 8 will underestimate the true variance of $\hat{S}_2$. For catch and escapement figures are themselves estimates, aging is subject to error, and non-Bristol Bay fish were present at Adak, although in small numbers. These additional sources of error cannot be adequately measured. However, a purely subjective evaluation of their combined effects would place a more realistic confidence interval for S_2 perhaps 20% wider than estimated previously.

A potential source of bias has not been mentioned — dropout mortalities in the gillnet fisheries.[6] Implicit in the model presented was the assumption that all deaths due to fishing could be measured by the catches in the high-seas and inshore fisheries, even though dropout deaths from these fisheries may have been a significant (but unknown) addition to mortalities due to fishing. But if the immature catch in year $i+1$ were relatively small, as in the present application, and two assumptions could be reasonably met, it can be shown that the occurrence of dropout mortality would not seriously bias the estimate of S_2.

These two assumptions are: (1) the rate of total exploitation by both the high seas and the inshore fisheries is the same for the 2-ocean mature run in year i as for the 3-ocean run in year $i+1$;[7] and (2) the ratio of dropout deaths to catch is the same in the two fisheries and for the two ocean ages. On the strength of these two assumptions, any measured run, R, of either 2- or 3-ocean mature sockeye can be related to the actual run, R′, which includes the correction for dropout deaths, by the simple expression $R' = cR$ where c is a constant greater than 1.

Now, assume the Japanese immature catch was zero in 1965 (it was relatively small). When $R_{s.i+1}$, $R_{s.i+1}$, and $R_{s.i+2}$ are replaced with the corrected

[6]Dropout deaths are those fish which become free of the nets before capture but which die as a result of entanglement with the gear.

[7]Although this rate was slightly higher for 3-ocean fish in 1966 than for 2-ocean fish in 1965, it was approximately 50% for both groups of fish.

runs, $cR_{x.i+1}$, $cR_{z.i+1}$, and $cR_{z.i+2}$ in equations 3, 4, and 5 it is seen that equation 5 should have given a relatively unbiased estimate of S_2 even with significant dropout mortality present:

$$\widehat{(N_{z.i}S_1)}' = \frac{cR_{x.i+1} - c\hat{p}_2 R_{z.i+1}}{\hat{p}_1 - \hat{p}_2} = c\widehat{N_{z.i}S_1}$$

$$\hat{N}'_{z.i+1} = c\widehat{N_{z.i}S_1} - cR_{z.i+1} = c(\widehat{N_{z.i}S_1} - R_{z.i+1})$$

$$\hat{S}'_2 = \frac{cR_{z.i+2}}{c(\widehat{N_{z.i}S_1} - R_{z.i+1})} = \frac{R_{z.i+2}}{\widehat{N_{z.i}S_1} - R_{z.i+1}} = \hat{S}_2.$$

It can be shown algebraically that with a large immature catch equation 5 would give an underestimate of S_2 according to the assumptions developed regarding dropout mortality.

The estimate of ocean survival presented in this paper, .582, is lower than the recent estimates arrived at by Parker (1962) and Fredin (1965). Parker suggested that the average annual survival rate in the ocean was relatively constant for the Pacific salmons and averaged .73. Fredin's estimates of the annual survival rate of 3-ocean sockeye from Bristol Bay and Karluk during their last year of life were .805 and .718, respectively. Fredin derived these two figures by independent means. The estimate in this paper suggests that the rate of ocean mortality of sockeye salmon, and more generally of all the Pacific salmons, may be more variable from year to year than previous estimates would imply, even during the period of life when the fish are relatively old and large.

It should be pointed out that the average rate of instantaneous natural mortality that was calculated is an adequate measure for short-term calculations only if natural mortality was relatively constant during the last 12 months at sea. An estimate of S_2 alone, no matter how precise, would provide no indication of a sharp change in the mortality rate during the last year at sea. For example, if the mortality rate increases sharply during the last month or so at sea as the fish migrate inshore, as has often been hypothesized but never demonstrated, then the estimate of M presented in this paper would be too great for the first 10 or 11 months preceding inshore migration and too low for the last 1 or 2 months of life.

ACKNOWLEDGMENTS

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Ecology of Phytoplankton in Lake Belwood, a Storage Reservoir in Southern Ontario[1]

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ABSTRACT

A 16-month study on a storage reservoir explained some of the factors controlling the seasonal development of phytoplankton in such an environment. Thermal stratification was unstable but was established in one of the two summers of the study and was associated with oxygen depletion in the hypolimnion. Both nitrate and phosphate concentrations showed distinct seasonality and the amounts in the influent were very similar to the amounts in the effluent. Large populations of *Cryptomonas* spp. developed in the early summer, when amounts of phosphate and nitrate were minimal, and were followed by a succession of planktonic Cyanophyta lasting into the winter. A population of *Peridinium aciculiferum* (Lemm.) Lemm. occurred over the winter. Based on their ecology in the reservoir the species of phytoplankton were divided into three groups: an autochthonous group, which developed populations simultaneously all over the lake; an allochthonous group, which sometimes formed populations around the inflow; and the river plankton, which rarely developed in the reservoir.

INTRODUCTION

An important means of controlling flood-water and conserving public water supplies is the construction of storage reservoirs on rivers and streams. Although the biology of these impoundments and the influence of the reservoir on the habitats downstream have been the subject of several studies, comparatively little is known of the ecology of phytoplankton in such environments. Wright (1954) in his investigation of the hydrobiology of Atwood Lake, a flood-control reservoir in Ohio, discusses the general features of the seasonal distribution and abundance of phytoplankton in relation to several factors. Brook and Rzoska (1954) studied the plankton in and below a large reservoir on the White Nile in Sudan, and Rodhe (1964) reported on the water chemistry, plankton, and productivity of a newly impounded lake in Swedish Lappland. In Czechoslovakia, Javornicky (1966) has investigated the seasonal dynamics of the phytoplankton of Slapy reservoir, and in the USSR recent research on the development and productivity of phytoplankton in reservoirs includes that of Prijmatchenko (1961) and Salmanov (1961).

Features common to many storage reservoirs include a large seasonal variation in water level, periods of turbidity, a large discharge for at least a part of the year, density of other currents induced by the inflow–outflow, and often complex or unstable conditions of thermal stratification largely controlled by the temperature and volume of the inflowing stream and the

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position and volume of the outflow. The present study is concerned with the seasonal development of phytoplankton populations in a storage reservoir on a calcareous river in southern Ontario in relation to the above features and also to some chemical factors and the algal populations in the inflowing river.

Lake Belwood (Fig. 1) was created in 1942 by the construction of the Shand Dam across the Grand River about 4 km to the north of Fergus. The capacity of the reservoir is 59,206,348 m^3 and the maximum depth is 22 m. The mean annual inflow would fill the reservoir in 50 days. Each year the

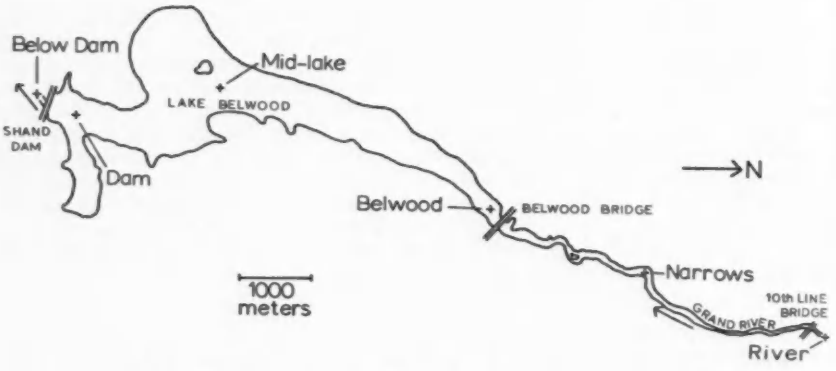

FIG. 1. Lake Belwood showing the positions of the six sampling stations.

reservoir is slowly drained during the late summer and autumn and is kept at or near the minimum over the winter. The reservoir fills rapidly with the spring thaw in March and early April, and the maximum level is maintained for part of the summer (Fig. 2). The flow in the Grand River above the lake at the 10th line bridge is usually minimal during the summer, and the highest rate of flow, and thus the great bulk of the yearly volume of water, occurs over the period December–March inclusive. About 11 km upstream of Lake Belwood is Luther Lake, another storage reservoir, the outlet of which is a tributary of the Grand River. Luther Lake is over twice the area of Lake Belwood but is shallow and has only one-fifth of the volume. Luther Lake is highly productive and large crops of phytoplankton, mainly Cyanophyta, are produced each summer and autumn. The overflow from Luther Lake is from the top of the dam, and it carries large numbers of phytoplankters into the Grand River and eventually into Lake Belwood. There is generally some degree of overflow all year round. Each year, in mid June, the dam is lowered by 0.6 m (2 ft) and the resulting discharge is usually complete by August. Between Luther and Belwood lakes the Grand River flows through farming land and two small towns.

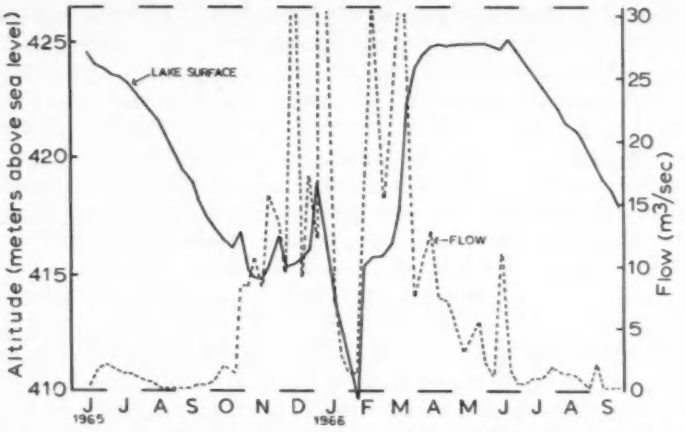

FIG. 2. Variations in the altitude of the surface of Lake Belwood and the average weekly rate of flow of the Grand River at the 10th line bridge from June 1965 to September 1966.

Two other studies were conducted on the biology of the Grand River concurrent with the one reported on here. Fraser (MS, 1966) described the seasonal growth and distribution of algae in the Grand River, and Spence (MS, 1967) investigated the influence of Lake Belwood on the chemistry and fauna of the river.

PROCEDURES

Six sampling stations were selected (Fig. 1). Four stations were in Lake Belwood, one was at the 10th line bridge, and the other just below the dam. The dam and the mid-lake stations were always lentic, but as the reservoir was drained in the autumn, first the narrows station and then the Belwood Bridge station became lotic. At the minimum level in February 1966 the ice-covered lake appeared to extend from the dam to a point about half way between the mid-lake station and Belwood Bridge.

Samples of water for chemical analysis and phytoplankton enumeration were collected weekly or fortnightly from June 1965 to September 1966. At the start every station was sampled, but this proved unnecessary and sampling for chemical analysis was restricted to the 10th line bridge, the dam, and below the dam. Samples for phytoplankton enumeration were collected from the 10th line bridge, Belwood bridge when the lake extended there, above the dam, and below the dam. No samples of any kind were collected from Belwood bridge from mid October 1965 to April 1966.

Samples from within the lake were collected at a depth of 1 m. All samples were kept in polyethylene bottles and the chemical analyses were begun on the same day as collection whenever possible. *p*H was measured in the laboratory using a Fisher Accumet *p*H meter. Alkalinity expressed as milligrams per

liter of $CaCO_3$, nitrate nitrogen, and silicate were measured by the methods in Mackereth (1963). Total phosphate phosphorus was measured by a slightly modified method from Standard Methods (American Public Health Association, 1965). From June to September 1966 only, total iron was measured by the 2,4,6-tripyridyl-s-triazine (TPTZ) method in Hach (1965). All water for analysis was filtered through Whatman No. 42 paper except that water for iron analysis was filtered through a membrane filter with a 3-μ pore. In an experiment to determine the size distribution of particulate phosphate a variety of filters were used on water samples collected in August 1966 from the 10th line bridge, the dam, and below the dam.

The populations of phytoplankton were estimated by the sedimentation of 25-ml, 50-ml, or 100-ml samples with Lugol's iodine followed by counting with an inverted microscope (Lund et al., 1958). Units were a single cell, a colony, or a filament 450 μ long (i.e. the diameter of the field of the Whipple eyepiece used). The counts were expressed as units per milliliter and are plotted on a semilogarithmic scale.

At intervals during both summers of the investigation temperature and oxygen profiles were taken at all stations using an Electronic Instruments Limited dissolved oxygen meter and thermistor with a 30-m (100-ft) cable. Lake level and flow data were supplied by the courtesy of the Grand River Conservation Authority.

RESULTS

Temperature and Oxygen

On June 22, 1965 there was little thermal stratification at any of the stations (Fig. 3). The temperature of the river water at the 10th line bridge was 27 C and the temperature of the surface water of the lake stations was around 20 C. The dam station showed the greatest range in temperature, from 19 C at the surface to 13 C at 19 m, near the bottom. At the narrows station a temperature range of 4 C existed over the 22 m depth of water. At all four of the lake stations the per cent saturation of oxygen decreased with depth. The lowest value recorded in the lake was 40% saturation at the narrows. Supersaturation of oxygen near the surface existed at the dam, mid lake, and Belwood bridge stations.

The profiles of July 20 showed little change. With the exception of the dam station the lake was practically isothermal. At the dam station a difference of 5 C existed between 1 m and the bottom. The dissolved oxygen curves at the dam and mid-lake stations were similar to those of June 22 but at Belwood bridge and at the narrows the amount of oxygen at the bottom had increased with the reduction in the lake level.

On August 18 a slight thermocline was evident only at the mid-lake station; otherwise the lake was still unstratified thermally though a few degrees warmer than previously. At both the dam and mid-lake stations, dissolved oxygen was reduced near the bottom and the minimum amount was 30% saturation at the dam station.

On September 20, the lake was still practically isothermal but had cooled a few degrees from the previous readings. At both the dam and mid-lake stations a steep oxygen gradient existed over the bottom 2 or 3 m but the minima were similar to those of August 18. The remainder of the profiles were over 80% saturation.

In contrast to 1965, 1966 was a warm dry summer, especially June and July. The first measurements were made on July 4 and presented a vastly different picture (Fig. 4) from that for 1965. The surface waters were warm (27–28 C) and at the dam and mid-lake stations there was distinct thermal

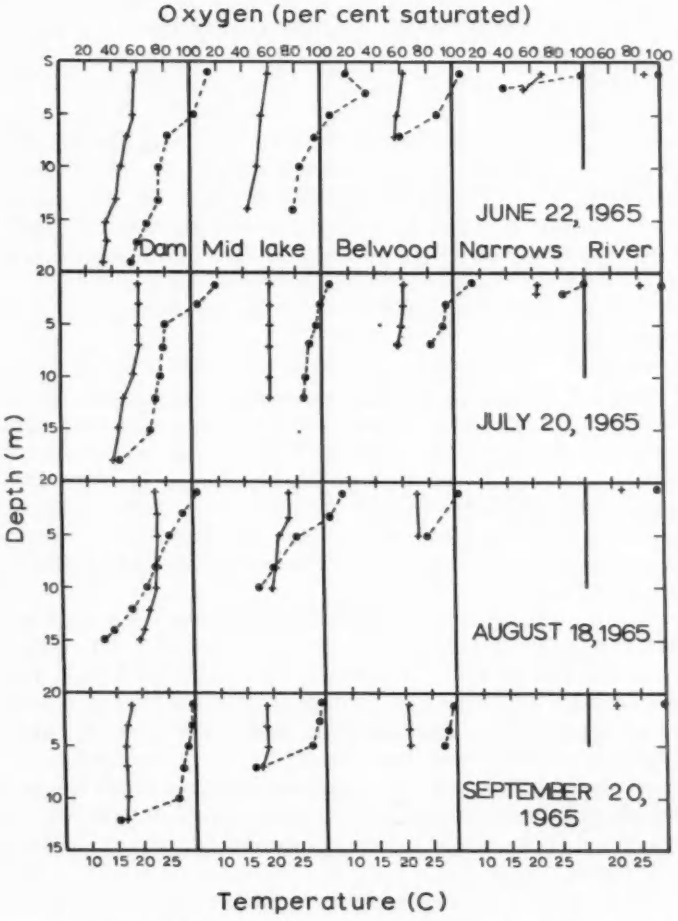

FIG. 3. Four series of temperature (+——+) and oxygen (●- - -●) profiles in Lake Belwood at five sampling stations in 1965.

stratification. The temperature difference at the dam station between 1 m and the bottom (19 m) was 15 C. By August 15th the thermal stratification had broken down in the lake though a colder, oxygen-deficient zone of about 3 m still persisted at the bottom of the dam station. Steep temperature gradients were also evident at the narrows station in the early part of the summer, as in 1965. At all four lake stations there was a decrease in oxygen with depth which was much steeper than in 1965. By July 18 there was no detectable oxygen at the bottom of the dam station and it was very low at the mid-lake station. On August 15 this oxygen gradient had broken down in both lake stations with the exception of the bottom 3 m of the dam station, where dissolved oxygen was still much reduced and was absent from the very bottom at 16 m. On September 13 an oxygen gradient had been re-established at the mid-lake station.

Chemical Analyses

The *p*H at all three stations had a mean of about 8.2 but during the study there was a distinct seasonal variation (Fig. 5). The highest values were in the summer. Only in the summer of 1966, when the lake was stratified, was there a consistent difference between the three stations. The highest values (up to *p*H 9.0) were at the 10th line bridge. The dam station was generally 0.2–0.3 of a unit lower and the water under the dam 0.5–1 unit lower. The alkalinity of all three stations varied little around a mean of 150 ppm $CaCO_3$.

The values for both nitrate and phosphate show a distinct seasonality over the period of investigation but, as with the *p*H, thre were no great differences between the stations. The level of nitrate rose from between 0.1 and 0.2 mg/liter in July at all stations to near 0.7 mg/liter in December above and below the dam, and to 0.5 mg/liter for the river at the 10th line bridge. Over the period December 1965–May 1966 the level of nitrate dropped slowly to between 0.1 and 0.2 mg/liter again in June and July. In August there was a temporary rise at all three stations but by September the level fell to below 0.1 mg/liter.

The seasonal variations in levels of total phosphate phosphorus show trends similar to those of nitrate. A minimum of less than 0.01 mg/liter was found in the summer of 1965. Generally higher amounts were found at all stations over the period from October 1965 to May 1966. Minimum amounts were recorded again in June 1966 but in August there was a rise to near the winter levels.

The amounts for total iron from June to September 1966 (Fig. 6) ranged from 0.01 to 0.18 mg/liter with most of the higher values in July. The analyses on water collected above the dam were consistently lower than those for the other two stations.

In the experiment to determine the distribution of particulate phosphorus (Table I), almost all of the total phosphate phosphorus detected by the method used passed through the 8-μ membrane filter. On the other hand only between

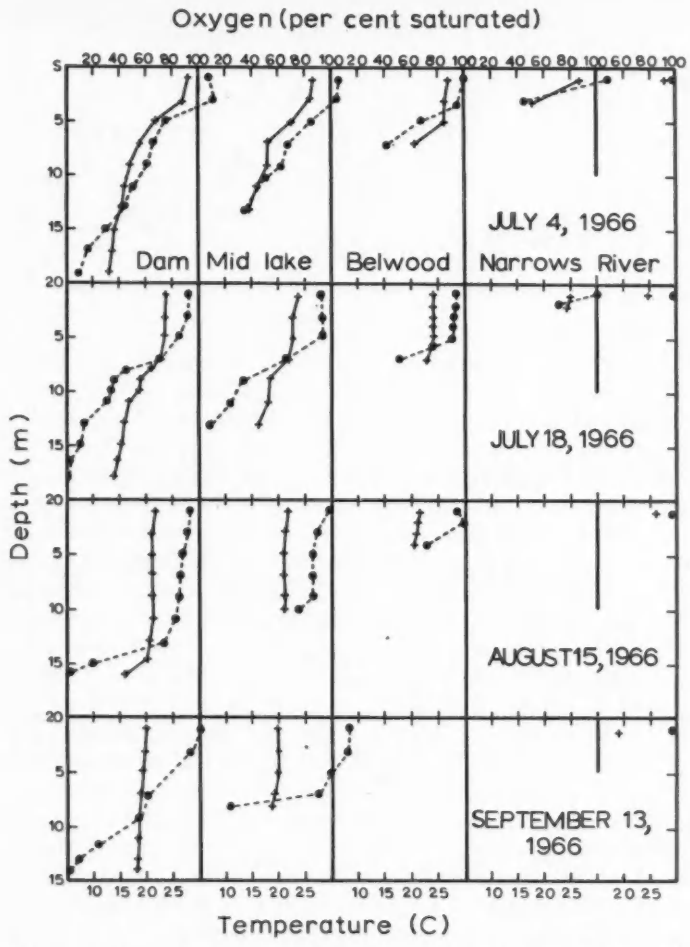

FIG. 4. Four series of temperature (+———+) and oxygen (●- - -●) profiles in Lake Belwood at five sampling stations in 1966.

one-quarter and one-tenth of this amount passed through the 10-mμ filter. The greatest difference was shown by the water collected at the 10th line bridge, where 0.019 mg/liter phosphate phosphorus was found in the water filtered through an 8-μ filter, and only 0.002 mg/liter in water filtered through a 10-mμ filter.

THE PHYTOPLANKTON

From the start of observations large populations of *Cryptomonas curvata* E. and *C. erosa* var. *reflexa* Marrson were recorded from the lake stations

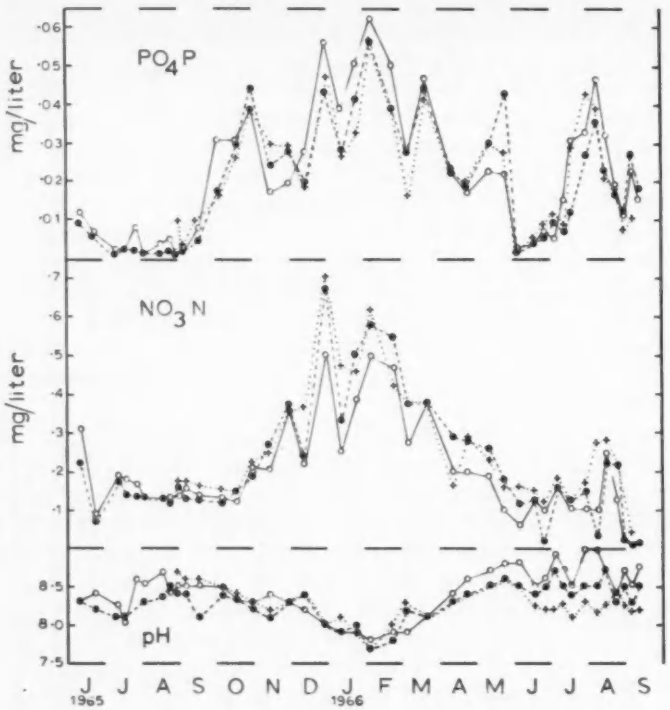

FIG. 5. The variations in pH, nitrate nitrogen, and phosphate phosphorus at the 10th line bridge (○——○) and dam (●– – –●) stations from June 1965 to September 1966, and below the dam (+.....+) from August 1965 to September 1966.

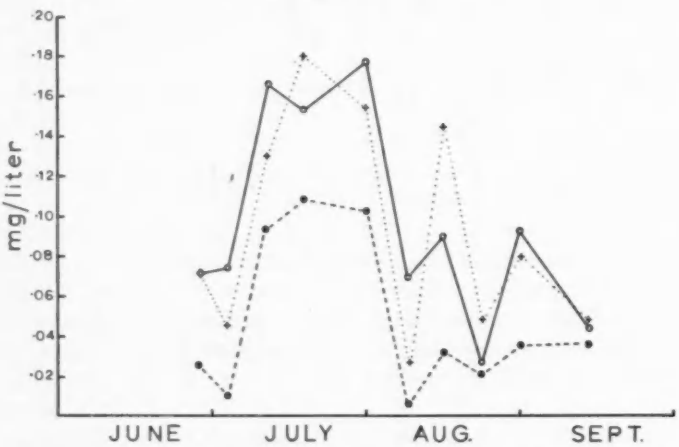

FIG. 6. The variations in total iron from June to September 1966 at the 10th line bridge (○——○) and dam (●– – –●) stations and below the dam (+.....+).

TABLE I. Concentrations (mg/liter) of total phosphate phosphorus in the filtrates of a series of millipore membrane filters and Whatman No. 42 paper.

	Filter				
Sample	10 mμ	0.22 μ	0.8 μ	8 μ	Whatman No. 42
10th line bridge	0.002	0.005	0.016	0.019	0.019
Dam station	0.005	0.008	0.015	0.016	0.016
Below the dam	0.002	0.003	0.015	0.016	0.017

(Fig. 7). The highest numbers of *C. curvata* were with few exceptions always at the dam station but this was not true of *C. erosa* var. *reflexa*. From populations of around 4000 cells/ml in the summer of 1965 *C. curvata* slowly declined in numbers through the fall of 1965 to 100 cells/ml in December. It was found in samples throughout the winter. An increase of up to 1000 cells/ml occurred in the spring and summer of 1966 but the maxima were never as large as in 1965. *Cryptomonas erosa* var. *reflexa* displayed maxima of around 1000 cells/ml at both Belwood bridge and above the dam in both summers, and was also found in samples throughout the winter. Both species were found in samples taken at the 10th line bridge but always in numbers below 100 cells/ml. Both species were usually found in samples taken below the dam but in 1965 only from October, when the lake level had dropped 7 m from the maximum, were there over 100 cells/ml of either. In 1966 *C. curvata* reached over 100 cells/ml below the dam in September.

During the winter of 1965–66 large populations of *Peridinium aciculiferum* (Lemm.) Lemm. (*Glenodinium aciculiferum* (Lemm.) Lindem.) were recorded at all three stations (Fig. 7). For most of the period the lake and river above the dam were frozen but the station under the dam was never found to be entirely frozen over. A maximum of nearly 7000 cells/ml occurred above the dam in March 1966. The population curves for the three stations are not synchronous, the counts from the 10th line bridge especially being very variable from sample to sample. Both before and after the period of growth from December 1965 to May 1966, *P. aciculiferum* was rarely found.

During the summer and fall of 1965 and again during the summer of 1966 large growths of *Aphanizomenon flos-aquae* (L.) Ralfs, *Microcystis aeruginosa* Kuetz., emend. Elenkin, and a species of *Oscillatoria* occurred (Fig. 8).

At the dam station the population of *A. flos-aquae* first exceeded 100 filaments/ml in July 1965 and maintained a population of between 1000 and 7000 filaments/ml through October. By mid November the population had dropped to less than 100 filaments/ml. At Belwood bridge the period of growth began a little later and reached 500 filaments/ml in early September before declining rapidly as the lake receded. Counts greater than 100 filaments/ml

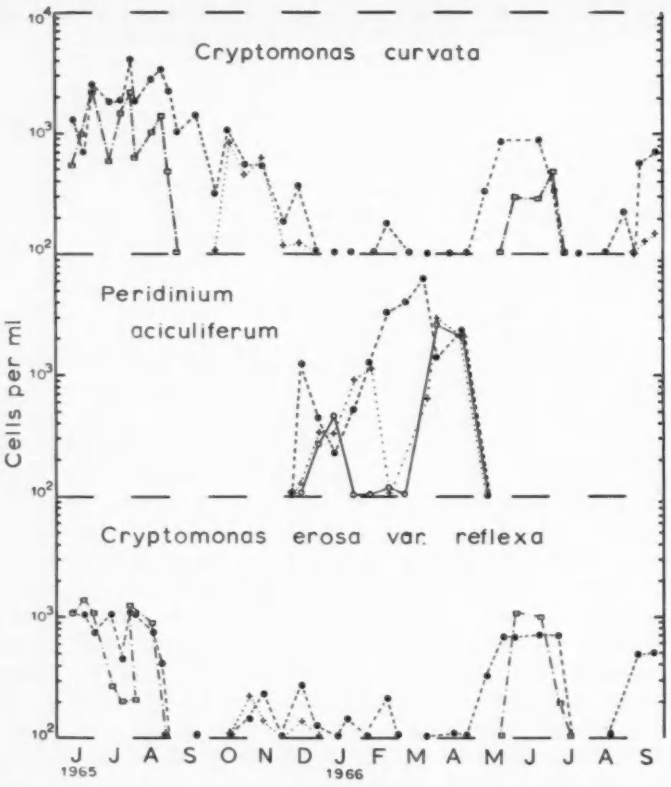

FIG. 7. The variations in populations of three species of phytoplankton from June 1965 to September 1966 at the 10th line bridge (○——○), Belwood bridge (□-.-.-□), and dam (●---●) stations, and below the dam (+....+).

were recorded from below the dam from September to November 1965 and in September 1966. In 1966 counts of over 100 filaments/ml were recorded first at Belwood bridge in July and a larger population was achieved at that station than in 1965. As in 1965 the population at Belwood bridge declined when the lake level receded. At the dam station in 1966 the maximum population up to the end of observations was about 3000 filaments/ml, considerably lower than in 1965. *Aphanizomenom flos-aquae* was never found at the 10th line bridge in 1965 but in 1966 was found there in low numbers in August.

In both summers large numbers of a species of *Oscillatoria* close to *O. agardhii* Gomont occurred in the plankton of the reservoir. At the dam station populations greater than 100 filaments/ml existed from June 1965 to January 1966 and from July to the end of observations in 1966. In 1965 there was a maximum of about 3000 filaments/ml in September and one of about 4000

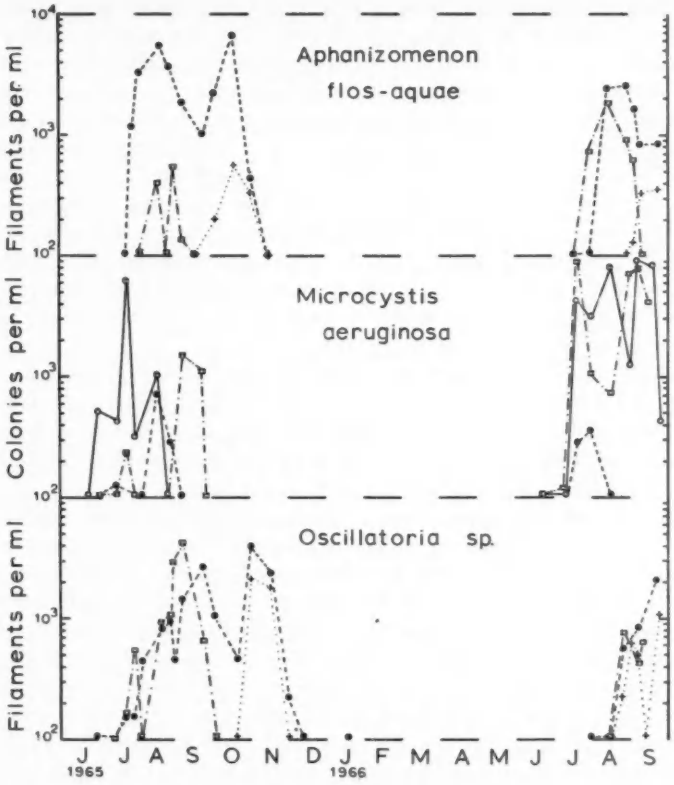

FIG. 8. The variations in populations of three species of phytoplankton from June 1965 to September 1966 at the 10th line bridge (○——○), Belwood bridge (□-.-.-□), and dam (●- - -●) stations, and below the dam (+....+).

filaments/ml in November, both at the dam station, and in 1966 the population had reached 2000 filaments/ml at the dam when observations ceased.

At Belwood bridge there was a maximum in 1965 of just over 4500 filaments/ml in early September, just prior to the maximum at the dam. The numbers then declined with the falling water level. In 1966 the population at Belwood bridge reached about 800 filaments/ml in August.

Below the dam, *Oscillatoria* sp. first reached over 100 filaments/ml in mid October 1965, although it was found in low numbers throughout the summer. A maximum of over 2000 filaments/ml was reached by the end of October, then numbers declined to less than 100 filaments/ml early in December. In 1966 *Oscillatoria* sp. reached over 100 filaments/ml below the dam much earlier, in August.

Unlike the other two abundant blue–green phytoplankters, in both years large populations of *M. aeruginosa* entered the lake via the Grand River. In both years the occurrence of large numbers of *M. aeruginosa* in the Grand River coincided with the release of water from Luther Lake in mid June. The patterns of development differed between the two years. In 1965 populations of over 100 colonies/ml existed at Belwood bridge and above the dam from late June to September but the major growth at Belwood bridge took place in September. By then *M. aeruginosa* had become a rarity in the river at the 10th line bridge. The maximum reached at Belwood bridge was about 1000 colonies/ml in early September, the numbers dropping to 100 colonies/ml by the end of the month with the receding lake level. At the dam station the population reached 700 colonies/ml in mid August 1965 but fell to less than 100 colonies/ml by the end of the month.

In 1966 populations of near 10,000 colonies/ml were recorded at times from July to September in the Grand River at the 10th line bridge and at Belwood bridge. No such large development took place above the dam, where a maximum of 400 colonies/ml occurred at the end of July before a rapid decrease. In both years few colonies were noted below the dam.

Several other planktonic species were observed in Lake Belwood but never reached the importance of the six species that have been described. The most important were *Anabaena flos-aquae* (Lyngb.) De Breb., *Coelosphaerium naegelianum* Unger, *Lyngbya birgei* G. M. Smith, and *Asterionella formosa* Hass. *Anabaena flos-aquae* was present in low numbers throughout 1965 at Belwood bridge and above the dam and was also frequent, though never abundant, in samples from the 10th line bridge. In 1966, however, a period of rapid growth took place in August at both Belwood bridge and above the dam, the populations reaching 3100 colonies/ml and 1200 colonies/ml respectively. The population then declined rapidly and was below 100 colonies/ml at both stations when observations ended. *Coelosphaerium naegelianum* was present in low numbers at all stations in both summers. *Lyngbya birgei* was found only at the dam station, in low numbers in both summers. *Anabaena formosa* was present at the dam station in numbers of around 700 colonies/ml in June 1965 and around 500 colonies/ml in June 1966 but otherwise was a rarity. It was never found in the river or at the 10th line bridge.

DISCUSSION

An important feature of Lake Belwood, or any storage reservoir, is that there is a variable but often considerable inflow and discharge which must influence all aspect of the hydrobiology of the lake. During the winter, when the reservoir is nearly empty, there is practically an unrestricted flow-through; but during the summer, with a high water level and little discharge, the reservoir is very similar to a natural lake with the important exception that the outflow is from the bottom of the dam. Under such conditions, thermal stratification is often of an unusual nature and is unstable.

Lake Belwood never stratified thermally in the summer of 1965 but in 1966 a form of thermal stratification had developed by July 4 and persisted for part of the summer. The rather atypical form of thermal stratification and the weak thermocline in the summer of 1966 may be explained by the conditions in the reservoir. All through the summer the inflowing river water mingles with the epilimnion. At the same time the only water leaving the reservoir is the cold hypolimnion. These factors together with the influence of the currents induced by the discharge are probably responsible for the peculiar thermal conditions in the reservoir and also for the instability of stratification. The much greater hypolimnetic oxygen deficiency in the summer of 1966 than in the summer of 1965 was a direct result of the degree of thermal stratification.

A cold-water density current, where a cold inflow passes straight through the reservoir, probably does not occur in Lake Belwood, at least during the summer months. In the winter the reservoir is drained and during periods of thaw a large inflow–outflow occurs under the ice. Dendy and Stroud (1949), Pfitzer (1954), and Stober (1963) in their work on storage reservoirs all reported the presence of cold-water density currents which were largely responsible for unusual temperature and oxygen profiles.

The thermal stratification in Lake Belwood in the summer of 1965 was considerably different from that in 1965 and it probably varies from year to year. A complex series of relationships exists between the volume and temperature of the inflow, the thermal conditions in the lake, the volume and position of the outflow, and the prevailing weather. All aspects of the physics, chemistry, and biology of the lake are influenced by these relations so it is not surprising that compared with a natural lake the productivity of a storage reservoir varies from year to year. The standing crop in 1965 was, on the average, greater than in 1966 (Fig. 7 and 8). The primary productivity of Lake Belwood has not yet been investigated. In 1965 the average secchi disc transparency at the dam and mid-lake stations was 2.5 m, in 1966 with thermal stratification, it was 4 m. The reservoir is full each year by early April and for a period of a few weeks it contains very few phytoplankton.

The seasonal variations in amounts of total phosphate phosphorus and nitrate nitrogen in the Grand River were also observed by Fraser (MS, 1966) and are in good agreement with the findings here. The curves for the river above the lake at the 10th line bridge, in the lake, and immediately below the dam were very similar (Fig. 5), though with the phosphate there was a tendency for the figures from the 10th line bridge to be highest in the winter months and with the nitrate for those from the 10th line bridge to be lowest in the winter months. There was also a tendency for nitrate to be higher under the dam than in the lake. Spence (MS, 1967) compared inorganic phosphate levels above and below Lake Belwood and suggested that the similarity is caused by a balance of utilization of phosphate in the surface waters with release in the hypolimnion during conditions of de-oxygenation. Rodhe (1964) demonstrated that an increase in the supply of nutrients to an impounded

lake was more obvious in terms of increased productivity than in the environmental conditions. Chalupa (1957) compared the levels of phosphate in the influent and effluent streams of a Czechoslovakian reservoir and concluded that utilization and release of phosphate in the reservoir were balanced and so the amount in the effluent was the same as the amount in the influent. Spence (MS, 1967) also investigated the fate of nitrate in Lake Belwood and suggested that the slightly higher levels under the dam may be due to nitrogen fixation by Cyanophyta or to the action of nitrifying bacteria or both. The increase in the levels of both phosphate and nitrate at all stations in late July and August 1966 was unusual. The increase was coincident with the occurrence of a period of rain after a long dry spell and also with the breakdown of stratification in Lake Belwood.

In spite of the alkalinity of the water there appeared to be no deficiency in iron. Of interest is that the figures for the dam station were consistently lower than the figure for the 10th line bridge or below the dam. The iron is probably in particulate or combined form and presumably some settling takes place. It may be significant that the greatest difference between the dam and the other stations occurred in July 1966, when the conditions above the dam were most lake-like. On the other hand the experiment to determine the size distribution of particulate phosphate (Table I) showed that there was more phosphate above the dam (dam station) in the 0.22-μ and 10-mμ filtrates than in the influent (10th line bridge) or effluent (below the dam).

The ecology of the three major species of planktonic blue–green algae in Lake Belwood presents some interesting differences. In the summer of 1965 the largest growths of *A. flos-aquae* took place at the dam station; the populations at Belwood bridge were always lower (Fig. 8). This was not the case in 1966, when about equal growth took place at the two stations. In 1965 large maxima of the *Oscillatoria* sp. occurred at both Belwood bridge and at the dam station and the periods of growth, but not the maxima, were concurrent with those for *A. flos-aquae*. The maximum of *A. flos-aquae* that occurred in mid August 1965 at the dam station took place when the population of *Oscillatoria* sp. was under 1000 filaments/ml, and the maximum of *Oscillatoria* sp. in late September coincided with a temporary minimum of *A. flos-aquae*. The largest maximum of *A. flos-aquae*, in October, coincided with a minimum of *Oscillatoria* sp. Finally, when the population of *A. flos-aquae* was declining in late October and November another maximum of *Oscillatoria* sp. occurred. The effect was not so marked at the Belwood bridge station but in 1965 the standing crop of *A. flos-aquae* was never very large. In 1966 the effect was again observed at the dam station but less strongly so at Belwood bridge station. Thus it appears either that large populations of *A. flos-aquae* and *Oscillatoria* sp. are mutally exclusive or that some undetermined environmental factor was responsible. Both species appeared in numbers greater than 100 filaments/ml under the dam only when the level of the reservoir was 6 or 7 m below the maximum.

In both years large populations of *M. aeruginosa* were observed in the river above the lake at the 10th line bridge. This is in contrast to the other two species of planktonic Cyanophyta. In 1965 moderate standing crops were observed at both Belwood bridge and above the dam but in 1966 the standing crop at Belwood bridge was very large and reached nearly 10,000 colonies/ml in July and again in September before the lake receded from the bridge. *Microcystis aeruginosa* was rarely found below the dam. The reason for the large crop of *M. aeruginosa* at Belwood bridge in 1966 and not in 1965 is not obvious.

The flagellates *C. curvata* and *C. erosa* var. *reflexa* were found in almost every plankton sample from Lake Belwood. Both species were present in large numbers in June 1965 at the beginning of observations. As with the planktonic Cyanophyta, the populations declined with the lowering of the water level at Belwood bridge and large numbers appeared below the dam only in the late summer when the level of the lake was sufficiently low. In both 1965 and 1966 the largest populations of both flagellates were associated with the minima of phosphate and nitrate, and the gradual decline in numbers in the fall of 1965 corresponded with an increase in phosphate and nitrate. The unusual increase in phosphate and nitrate that occurred at all stations in July and August 1966 coincided with a very distinct minimum of both flagellates.

The only alga that was present in appreciable numbers over the winter of 1965–66 was *P. aciculiferum*, which occurred throughout the system. The population varied with the intermittent thaws but a general maximum may be discerned in March especially at the dam station. For most of the time the growth of *P. aciculiferum* took place under a cover of ice and snow. The populations declined in April while the lake was filling with the spring runoff and by early May it was a rarity.

Considerable numbers of epiphytic and epilithic diatoms entered the lake with the spring runoff but these never multiplied and rapidly disappeared. They included species of *Meridion*, *Diatoma*, *Gomphonema*, *Fragilaria*, and *Cymbella*. In June 1965 a small population of *A. formosa* was present at the dam station only. However, the population declined rapidly and apart from another small increase in 1966, *A. formosa* was a rarity in plankton samples. Colonies of *Fragilaria capucina* Desm. and *F. crotonensis* Kitt. were often found in plankton samples in both summers mainly at the dam station but occasionally at Belwood bridge. In both years the major population in the lake in spring was of *Cryptomonas* spp. *Cryptomonas* spp. also dominated the spring growth in the Slapy reservoir in Czechoslovakia (Javornicky, 1966). Following the growth of the flagellates and overlapping with it were the large populations of Cyanophyta which lasted in 1965 well into the fall. The variable populations were confined to the top few meters and only when the water level was low did appreciable numbers appear in the river below the dam.

Based on their ecology the phytoplankters of Lake Belwood may be divided into three groups. The first, an autochthonous group, includes *C.*

curvata, *C. erosa* var. *reflexa*, *Oscillatoria* sp., *A. flos-aquae*, and *A. formosa*. These formed populations at the dam station or simultaneously all over the lake proper, which extends to a little beyond Belwood bridge when the lake is full. They were rarely or never found in the inflow. The second, an allochthonous group, includes *M. aeruginosa*, *A. flos-aquae*, *A. spiroides* var. *crassa* Lemm., *Coelosphaerium naegelianum*, *Dinobryon sertularia* Ehr., and *D. divergens* Imhof. Large or significant numbers of these appeared from time to time in the inflow, some of them probably derived from Lake Luther. These may or may not have developed in Lake Belwood and sometimes formed populations at the upper end of the lake near Belwood bridge (e.g. *M. aeruginosa* in summer 1966). The third group comprises the river periphyton and phytoplankton brought into the lake with the inflow. These were mainly noticeable upstream from Belwood bridge in the full lake. Common examples are *Pediastrum boryanum* (Turp.) Menegh., *Scenedesmus* spp., *Fragilaria* spp., *Merismopedia* spp., and a great many other species of unicellular or colonial Cyanophyta and Chlorococcales. They very rarely formed populations of any consequence in Lake Belwood. This classification largely agrees with the conclusions of Javornicky (1966). On the basis of the phytoplankton ecology he divided the much larger Slapy reservoir into three regions, the upper or inflow region, the reservoir proper, and the elongated bays. Lake Belwood, on a much smaller scale, may also be described in this manner.

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Distribution of Transaminases (Aminotransferases) in the Tissues of Pacific Salmon (*Oncorhynchus*), with Emphasis on the Properties and Diagnostic Use of Glutamic-Oxalacetic Transaminase[1,2]

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ABSTRACT

A partially purified glutamic-oxalacetic transaminase (GOT) from salmon liver was shown likely to function best at a pH > 7.5, to be specific for the L isomer of aspartic acid, and to require pyridoxal phosphate for activity. The coenzyme appeared to be tightly bound to the apoenzyme.

A survey of the scope of apparent transaminations in dialyzed homogenates of brain, heart, kidney, liver, and muscle indicated that GOT and glutamic-pyruvic transaminase were active in all tissues and that α-ketoglutarate (α-KG) was a more widely used amino acceptor than pyruvate. No transamination to the amino acceptors α-KG or pyruvate could be detected using as donors β-alanine, D-alanine, γ-aminobutyrate, glycine, histidine, proline, serine, or threonine. The following amino acids showed various degrees of transamination with α-KG in at least some tissue homogenates: L-leucine, L-norvaline, L-ornithine, L-phenylalanine, L-tyrosine, and D-valine. Interpretation of these results is discussed, particularly in relation to comparative biochemistry.

Spectrophotometric estimations of plasma or serum GOT levels were used to distinguish at a significant level ($p < 0.01$), apparently healthy fish from those treated with the hepatic poisons bromobenzene and CCl_4 or those affected by bacterial kidney disease. The practical value of using GOT estimations as a diagnostic tool is discussed.

It is tentatively suggested from limited data that the activity (units of enzyme/milligram tissue N) of GOT in various tissues decreases in the following order: heart, liver, kidney, and muscle.

INTRODUCTION

"Transamination is a chemical reaction (Herbst and Engel, 1934) in which an amino group is transferred from one molecule to another without the intermediate participation of ammonia" (Meister, 1955). Transaminases (aminopherases, aminotransferases) are widely distributed, relatively specific enzymes which catalyze the general reaction

$$R_1CHNH_2COOH + R_2COCOOH \overset{B_6}{\leftrightharpoons} R_1COCOOH + R_2CHNH_2COOH$$

in which R_1 and R_2 represent carbon skeletons common to both amino and

[1]Received for publication January 16, 1968.

[2]The author has reluctantly used common or trivial names rather than systematic names in relation to transamination for ease of comparison with the bulk of the literature. The reader should be aware of the following recommendations of the International Union of Biochemistry (1964) concerning enzyme nomenclature. "Transaminases" should be termed "aminotransferases." "Glutamic-oxalacetic" or "glutamic-aspartic transaminase" should be termed, trivially, as "aspartate aminotransferase" and systematically, as "L-aspartate:2-oxoglutarate aminotransferase" (Enzyme Commission No. 2.6.1.1.). Similarly, "glutamic-pyruvic" or "glutamic-alanine transaminase" should be referred to as "alanine aminotransferase" and "L-alanine: 2-oxoglutarate aminotransferase" (EC No. 2.6.1.2.)

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keto acids and B_6 indicates catalysis by this B vitamin. This equation is actually the sum of two separate reactions which can be broadly expressed as

$$R_1CHNH_2COOH + [\text{pyridoxal enzyme}] \rightleftharpoons R_1COCOOH + [\text{pyridoxamine enzyme}]$$

$$[\text{pyridoxamine enzyme}] + R_2COCOOH \rightleftharpoons [\text{pyridoxal enzyme}] + R_2CHNH_2COOH$$

Transamination represents one of the principal metabolic pathways for the synthesis and deamination of amino acids. It is a reversible reaction and of broad scope (Cohen and Sallach, 1961; Guirard and Snell, 1964), involving, as it can in various tissues, all naturally occurring L-α-amino acids and their keto analogues, some aldehydes, amides (Meister and Tice, 1950; Meister, 1955), gamma and omega amino acids, a few monocarboxylic amino and keto acids, some D-amino acids (Hug and Werkman, 1957; Martinez-Carrion and Jenkins, 1965) and even compounds other than aminocarboxylic acids. Many transaminations are linked to the carrier pair glutamate-α-ketoglutarate (Cohen and Sallach, 1961) and less so to alanine-pyruvate (Rowsell, 1956b). In addition, ammonia can be incorporated indirectly into a variety of amino acids by the action of glutamic dehydrogenase on α-KG[2] to form glutamate which acts, as Dixon and Webb (1964) suggest, as a coenzyme or as a central depot for amino groups. Transamination therefore allows an interplay between carbohydrate, fat, and protein metabolism, an activity which can serve the changing demands of the organism. Although the relationships between transaminase activity and protein synthesis are still not clear (Smith and Williams, 1951), McAllan and Chefurka (1961a) found a close relationship between protein synthesis and transamination activity in some insects and Meister (1957) had previously suggested that the amino acid pool necessary for protein synthesis is regulated by transaminases. Guirard and Snell (1964) have reviewed the enzymology of transamination.

GOT levels in normal rat and human tissues increase in the following order: spleen, lung, testis, kidney, liver, brain, skeletal muscle, and heart muscle (myocardium) (Agress, 1959). According to Dubach (1957) GOT levels in the dog increase in a slightly different order, viz., lung, testis, brain, kidney, skeletal muscle, liver, and myocardium. In the latter series the myocardium had about twice the activity per unit wet weight of the liver. The high activity of the heart muscle induced Karmen (1955) to devise a simple, accurate assay for GOT and as a result to show a direct relationship between SGOT levels and acute transmural myocardial infarction in humans. In sera from normal patients levels ranged from 10–40 units/ml whereas in sera from patients suffering from acute myocardial infarction, the levels

[2]The abbreviations used are: GOT, glutamic-oxalacetic transaminase; PGOT, plasma GOT; SGOT, serum GOT; GPT, glutamic-pyruvic transaminase; SGPT, serum GPT; α-KG, α-ketoglutaric acid; NAD, nicotinamide-adenine dinucleotide; $NADH_2$, reduced NAD; NADP, nicotinamide-adenine dinucleotide phosphate; PLP, pyridoxal-5-phosphate; Tris, tris (hydroxymethyl) amino methane.

ranged from 100 to 6000 units/ml (see also LaDue et al., 1954). A further advantage of the test is that GOT levels of refrigerated sera remain constant for at least 2 weeks. The findings have been widely confirmed and extended to both positive diagnosis and to differential diagnosis by exclusion, of diseases such as hepatitis (Bang et al., 1959) and several others (Agress, 1959).

The possible physiological importance and diagnostic value of transaminases stimulated this exploratory investigation into the estimation, characteristics, and scope of some tissue transaminases in normal and diseased Pacific salmon. To my knowledge, this is the first report specifically on transaminations in fish[4] and I have, therefore, purposely discussed in some detail the comparative aspects of our results. Reviews by Gumbmann et al. (1958) and Tarr (1958) provide some useful background information on intermediary metabolism of fish.

MATERIALS AND METHODS

Reagents

All chemicals were of the highest purity commercially available. The chemicals and firms were as follows: amino acids (Mann and Calbiochem); α-KG, NAD reduced and malic dehydrogenase (Calbiochem); Na pyruvate (Sigma); and PLP (Nutritional Biochemicals).

Tissues and Homogenates

All samples from *Oncorhynchus* spp. were taken from cultured stocks which had been fed on artificial diets. Where possible blood was taken from the dorsal aorta using the abbreviated cannulation procedure of Smith and Bell (1964) or by severing the caudal fin, taking care that no external fluids contaminated the sample. When required, heparin was used as the anticoagulant and suspended matter was removed by centrifugation in the cold.

Brain — including pituitary; heart — atrium and ventricle; kidney — including "head" kidney and corpuscles of Stannius; skeletal muscle — near dorsal fin: white tissue only.

Homogenates were prepared by first excising the required tissue from exsanguinated, unsexed fish, blotting it dry, and storing it on ice or in the deepfreeze until needed. Tissues were then initially homogenized (1 g wet wt/4 ml, except skeletal muscle, 1 g/10 ml, the highest pipettable concentration) in ice cold 0.1 M *p*H 7.5 phosphate buffer for 3–5 min in an ice-cooled Potter-Elvehjem type device[5] and finally treated for 10 min in a cooled 20-kc Biosonic oscillator[6]. The homogenate was dialyzed for 16–18 hr against 0.01 M phosphate buffer at *p*H 7.5 (4 C) to remove endogenous amino and keto

[4]Note added in proof: The following reference has come to my attention: Creach, Y. 1967. Transamination entre les acides aminés et l'acide α-cetoglutarique chez la carpe. Arch. Sci. Physiol., **21**(4): 443–448.

[5]Tri-R Instruments Inc., Jamaica, New York.
[6]Will Scientific, Inc., New York.

acids (see Wood, 1958, for amino acid levels in sockeye). Homogenates were diluted 1:20–1:50 depending upon their activity, for spectrophotometric analyses.

Quantitative Estimation of GOT

GOT activity was measured according to the spectrophotometric method of Karmen (1955) which is based on the following coupled reactions:

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{L-glutamate} + \text{oxalacetate}$$
$$\text{oxalacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{malate} + \text{NAD}^+$$

excess
malic dehydrogenase.

The oxidation of reduced NAD was followed by recording the decrease in optical density at 340 mμ in a Beckman spectrophotometer. The procedure was usually as follows: to a 1-cm cuvette add 0.5 ml of 0.2 M L-aspartate (*p*H 7.5), 0.2 ml malic dehydrogenase (equivalent to at least 200 units), 0.1 ml reduced NAD in buffer (2 mg Na salt/ml), 0.2 ml of serum, tissue homogenate or enzyme preparation, and 2.0 ml of 0.1 M Sørensen's Na–K buffer *p*H 7.5. The solutions were thoroughly mixed and equilibrated for 10 min to a stable optical density after which 0.2 ml of 0.1 M α-KG, *p*H 7.5, was added, mixed, and the readings begun. The α-KG was omitted from the control system and the difference in volume made up with buffer. Oxidation of $NADH_2$ by serum alone — after the 10 min pre-incubation period — or in the presence of α-KG was negligible but there was oxidation in the presence of pyruvate and oxalacetate. Malic dehydrogenase appeared to be a particularly active endogenous component of salmon sera.

Temperature was stabilized, usually at 25 C, using Beckman thermospacers and the assays were done in duplicate. The difference in optical density units between the complete system and the control was attributed to GOT activity (Shepherd and McDonald, 1958).

One unit of transaminase activity is defined as the activity in 1.0 ml of sample which causes an optical density decrease of 0.001 in 1 min under the defined conditions. Units of enzyme activity were calculated from the linear portion ("initial velocity") of the enzyme progress curve. Activities calculated on the basis of differences between initial and final absorbancies measured over a fixed time period could be seriously lower than the actual values. Nevertheless such a basis has been used by many workers.

The proportionality between the amount of blood serum (i.e. GOT) and the rate of the reaction as well as the kinetics of the oxidation are shown in Fig. 1.

Qualitative Estimation of Transamination

A 0.5-ml aliquot of tissue homogenate, supplemented with 15 μg PLP, was added to a 15-ml centrifuge tube containing 100 μM of amino acid and

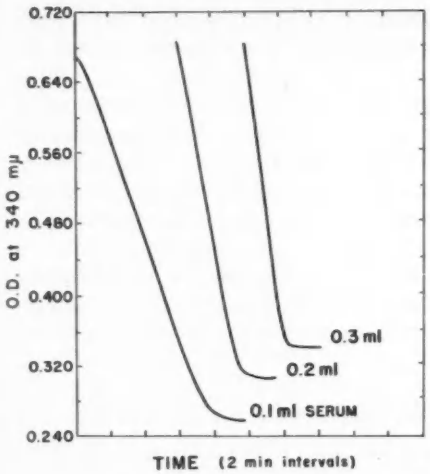


FIG. 1. Effect of varying amount of blood serum on the spectrophotometric assay for GOT. (Tracing from recorder attached to Beckman DU.)

20 μM of keto acid (both acids neutralized) and the mixture was made to 2.0 ml with 0.1 M phosphate buffer, *p*H 7.5. The qualitative estimations were also carried out using 20 μM each of amino and keto acid. Further, TES (N-Tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid) also proved to be an effective buffer and might have much to recommend it (Good et al., 1966). Control systems containing the amino and keto acids separately were used to correct for the formation of alanine or glutamate from pyruvate or α-KG respectively by mechanisms other than transamination. The possibility of nonenzymatic transamination was examined using boiled homogenate.

The tubes were incubated at 25 C for 1½ hr and the reaction was terminated by adding 2 ml of tungstic acid. (Just before use mix 10% sodium tungstate: 1N H_2SO_4: H_2O 10/10/30 v/v.) The precipitate was removed by centrifugation and the supernatant used for paper chromatography. It was discovered near the end of this work that such precipitation could usually be omitted and superior chromatograms, especially of tyrosine, could be obtained by spotting the reaction mixture directly on the paper.

Paper chromatography.— The amino acids were separated by spotting 30–40 μliters of the above supernatant on Schleicher and Schuell No. 507 paper and processing unidimensionally with methanol/water/pyridine (60/20/20 v/v) according to the ascending method of Redfield (1953). If the separation was unsatisfactory, the second dimension was run or as was frequently the case, the methanol/water/pyridine system was used as a descending solvent on Whatman No. 1 paper. Mixtures containing arginine and/or ornithine were best separated on Whatman No. 1 paper using an ascending solvent of ethanol/H_2O/diethylamine (77/22/1 v/v) (Block et al., 1958).

Induced and Natural Tissue Damage

In order to assess the diagnostic value of SGOT estimations in detecting tissue damage we attempted to inflict surgical injury to the heart and liver but this proved to be tedious and difficult to replicate. Chemically induced trauma of fasting fish was somewhat more successful although quantitative administration of substances to fish is often difficult and inaccurate because of leakage and regurgitation. Bromobenzene (150 mg/kg body wt) which is known to cause central hepatic necrosis within 2 days in fasting rats (see Williams, 1959), was injected intraperitoneally at three times this level.

An opportunity to examine the relationship between naturally induced tissue damage and SGOT levels was presented by the occurrence of an epizootic of bacterial kidney disease (Bell, 1961) in a stock of cultured sockeye (*O. nerka*).

Purification and Resolution of GOT

The main purpose of purification was to attempt to resolve the holoenzyme into apoenzyme and coenzyme and thus to establish the identity of the cofactor. Previous attempts based on dialysis of whole sera and photolysis (Schlenk et al., 1946) of the cofactor were unsuccessful. The problem is thoroughly discussed by Guirard and Snell (1964).

Liver (59 g) from a wild, adult, male coho (*O. kisutch*) was fractionated according to the procedure of McAllan and Chefurka (1961b) and the purified enzyme was resolved by use of their dialysis method except that we had to repeat the dialysis in 500 ml of 20% (w/v) $(NH_4)_2SO_4$ three times in order to eliminate enzyme activity. No attempt was made to estimate the efficiency of the purification procedure.

Estimation of Nitrogen

The titrimetric microkjeldahl method of Ma and Zuazaga (1942) was used essentially unmodified to estimate the amount of nitrogen in the tissues. A mercury rather than selenium catalyst was used to minimize the possibility of nitrogen loss (Hiller et al., 1948). The method gave a linear relationship between the amount of nitrogen and the volume of tissue homogenate over the range 0.25–1 ml at the usual concentration of 1 g tissue/4 ml buffer.

RESULTS

Effect of Temperature and *p*H on GOT Activity

The apparent optimum temperature for purified GOT, supplemented with PLP, was measured by following the rate of formation of oxalacetate as measured at 280 mμ according to the method of McAllan and Chefurka (1961a). This assay removes malic dehydrogenase as a complicating factor. Although no special significance should be attached to the values because they are the resultant of activating and inactivating factors (Dixon and Webb, 1964), they do give some idea of the operating range of the enzyme. Measurements

were further complicated by the slow flocculation of the enzyme preparation and the decomposition of oxalacetate at higher temperatures. The rate of reaction appeared to increase linearly from 10 to 30 C at *p*H 8.0 in Tris buffer but began to level off at 35–40 C, the highest temperature tested. The reaction was followed for 15 min.

Cook (1956) reviewed reports of *p*H optima of transaminases and found that the optima varied according to the source and type of enzyme. Reports of GOT optima in higher plants varied from 6.9 to 8.5 whereas in animal tissues values were close to *p*H 7.5. Evaluation of the data is difficult because many values were obtained using crude enzyme preparations under a variety of conditions. Lichstein et al. (1945) found that GOT of *Streptococcus faecalis* showed no distinct optimum over the range of 5.7–9.5 in phosphate buffers. McAllan and Chefurka (1961b), who suspected that anions were influencing *p*H optima, observed a similar response using purified roach enzyme in phosphate and Tris buffers. But they found a clear optimum at *p*H 8.3 in borate buffer which they attributed to complexing of the formed oxalacetate.

In this work transaminations were usually carried out at the customary *p*H of 7.5 in phosphate buffer for comparative purposes but the response of purified GOT to various reactions was tested. Results (Fig. 2) suggest that

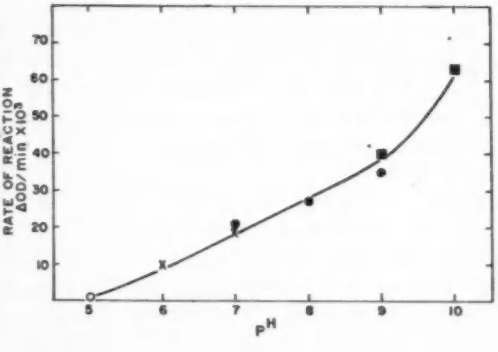


FIG. 2. Effect of *p*H on initial velocity (25 C) of purified GOT. Reaction mixture: 2.2 ml 0.1 M buffer, 0.2 ml 0.1 M α-KG, 0.1 ml enzyme supplemented with 0.75 μg PLP and 0.5 ml 0.2 M L-aspartate. Controls in which D isomer replaced L were used to correct for nonspecific effects of *p*H. Acetate (○), phosphate (×), Tris (●), and glycine (■) prepared according to Dawson et al. (1959).

there is no distinct optimum *p*H for GOT activity but the initial velocity is positively related to increasing *p*H, at least over the range 5–10. These results, although useful, must be viewed with some caution because there is also a tendency for the absorbancy of oxalacetate to increase with *p*H. This factor was apparently not taken into account by McAllan and Chefurka (1961b), nor were the possible anionic effects of various buffers considered by Cook (1956). Cook did point out that *p*H effects in this system are complex because four ionizable substrates — not to speak of the holoenzyme — are involved. However, experimental results using crude trout (*S. gairdneri*) liver homogenates and a glutamate assay also suggest that GOT is most active in the alkaline range (Bell, unpublished). The influence of *p*H on coenzyme activity,

i.e., the shuttling between aldehydic and amino forms, was discussed by Guirard and Snell (1964).

Specificity of GOT and GPT

In common with transaminases from most sources, our crude and purified GOT preparations were specific for the L isomer of aspartic acid, thus also suggesting the absence of an aspartate racemase. (D-alanine was also inactive in all tissue homogenates.) Enzymes specific for the D isomer have been studied in detail by Martinez-Carrion and Jenkins (1965).

No GPT activity could be detected in a purified, active preparation of GOT but strong GPT activity was found in the first $(NH_4)_2SO_4$ precipitate. This confirms the general observation that these are distinct enzymes.

Cofactor Requirement for GOT

All transaminases appear to require specifically either PLP or pyridoxamine-5-phosphate for activity (Braunstein, 1960): the function of these cofactors has been discussed by Guirard and Snell (1964) and Fasella (1967). Our resolved GOT was also activated by PLP (Fig. 3) but not by pyridoxal, pyridoxamine, or pyridoxine. These results were confirmed by chromatographic examination for glutamate.

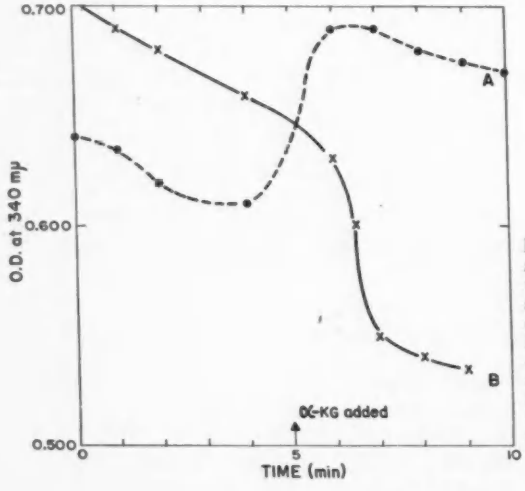


FIG. 3. Activation of GOT apoenzyme by pyridoxal-5-phosphate (PLP). Standard spectrophotometric assay except that (A) cuvette (●) contained 0.1 ml resolved rather than holoenzyme and (B) cuvette (×) contained 0.1 ml of resolved enzyme plus PLP (1 ml PLP(30 μg/ml) to 3 ml apoenzyme, incubated 15 min before using). PLP analogues were similarly tested but without effect (see text).

The weak activity of the purified enzyme might have been due to poor fractionation, insufficient preincubation of apoenzyme and coenzyme, excess of enzyme which bound and masked the oxalacetate and/or PLP (cf. "self inhibition" of lactic dehydrogenase (Straub, 1940)), or to the instability of

the enzyme preparation, as noted by Cammarata and Cohen (1950). The apparent slow activity of "resolved" enzyme might be attributed to residual PLP or oxidation of the reduced NAD by contaminants.

No attempt was made to determine if other cofactors were required because most published evidence suggests that PLP only is needed. The single exception to this seems to be the demonstration of an iron requirement for a legume GOT (Patwardhan, 1960) but this might have been at least partly due to an iron-chelate catalysis of nonenzymatic transamination (Guirard and Snell, 1964).

Enzymatic Synthesis of Amino Acids from Keto Acids

The scope of transaminase activity in various tissues was explored using the method outlined for qualitative estimation of transamination. The results presented in Table I indicate the wide distribution of both GOT and GPT and further, generally agree with the findings of Cammarata and Cohen (1950) and Cohen and Sallach (1961). The latter workers also had found (in pig tissue) that α-KG appeared to have broader scope as an amino acceptor than had pyruvate but more thorough, comprehensive testing of pyruvate might alter this conclusion.

Cohen's group found that in lyophilized aqueous extracts of pig tissues the following amino acids reacted with α-KG at decreasing rates: aspartate, leucine, valine, isoleucine, alanine, tyrosine, ornithine, and phenylalanine. These amino acids were also among the ones most frequently showing activity in salmon tissues. Neither our data nor those of Cammarata and Cohen (1950) permit quantitative comparison of apparent transaminase activity between tissues. Inter-tissue differences in activity might simply have been due to differences in protein concentration of the homogenates rather than to inherent differences in specific activities of the various tissues, i.e., units of enzyme activity/mg tissue protein. However, as will be discussed later, inter-tissue differences in specific activities of certain transaminases probably do exist in salmon (see Table VI).

Further reactions might have been clearly demonstrated if we had used more concentrated tissue extracts, as suggested by Cammarata and Cohen (1950) although concentration of muscle homogenates to $\frac{2}{3}$ volume did not help to reveal any other reactions. Our use of whole homogenates might have been advantageous because there could be qualitative and quantitative differences in activity between tissue fractions (Cohen and Sallach, 1961).

In general the results in Table I were consistent from species to species but valine, ornithine, and phenylalanine showed some variation in activity which might have been attributable to species, sex, age, residual blood in the tissue, nutritional or physiological condition, or other more subtle variables. For example, Rosen et al. (1958) found that hydrocortisone could greatly increase the GPT but not GOT activity in rat livers and Ogino (1965) showed that both GOT and GPT activity in carp was dependent upon the vitamin B_6 uptake of the fish (no PLP was added to the enzyme preparation).

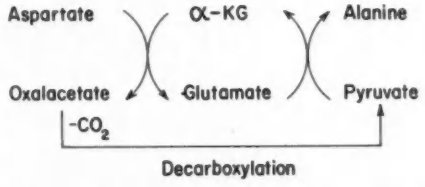
TABLE I. Apparent transaminations in whole, dialyzed homogenates of various tissues from unsexed, juvenile *O. gorbuscha*, *O. kisutch*, and *O. nerka*. (0, negative; +, positive; ±, weak or doubtful.)

	Net glutamate synthesis[a] (α-KG amino acceptor)						Net α-alanine synthesis[a,f] (pyruvate amino acceptor)[b]				
Amino donor	Liver	Kidney	Heart	Muscle	Brain	Red blood cells	Liver	Kidney	Heart	Muscle	Brain
L-Alanine	+	+	+	+	+	+					
β-Alanine	0	0	0	0			0	0	0	0	0
γ-Aminobutyrate	0	0	0	0	0		0	0	0		0
L-Arginine	+	+	0	0							
L-Aspartate[c]	+	+	+	+	+	+	0	0	0	0	0
L-Glutamate							+	+	+	+	+
Glycine	0	0	0	0	0	0	0	0	0	0	0
L-Histidine	0	0	0	0			0	0	0	0	0
L-Leucine	+	+	+	+	+	0		0	0		
L-Norvaline	±	0	+	±	+		±	0	0		0
L-Ornithine	+	+	±	±	+		±	+	0		0
L-Phenylalanine[c,d]	+	+	+	+	+	0		0			0
L-Proline[g]	0	0	0	0			0	0	0		
L-Serine	0	0	0	0			0	0	0	0	0
L-Threonine	0	±	0	0			0	0	0	0	0
L-Tyrosine[e]	+	+	±		+		0	0			+
L-Valine	±	±	+	+	+	0		0	0		0

[a]Indicates increase over homogenate with either α-KG or pyruvate alone.
[b]System contained 0.02 M NaN_3 to inhibit oxidation of pyruvate (Quastel and Witty, 1951).
[c]Reverse reactions also demonstrated.
[d]Reverse reaction demonstrated by Rowsell (1956a) with washed rat liver particles and no added PLP.
[e]Added as a saturated, neutralized solution.
[f]Some or all might be formed indirectly by coupling with GPT (see "Results").
[g]See Table II.

The mere demonstration of enzymatic transamination in crude homogenates does not necessarily imply the existence of a specific transaminase for two important reasons: purified transaminases so far studied show overlapping substrate specificities; coupling can occur between two different transamination reactions if the (donor) amino acid reacts with the pyridoxal form of one transaminase and the (acceptor) keto acid reacts with the pyridoxamine form of the second transaminase (Guirard and Snell, 1964; Rowsell, 1956b). The latter mechanism has been shown to account for apparent transamination between aspartate and pyruvate in pig heart extracts (O'Kane and Gunsalus, 1947). Traces of glutamate or α-KG can allow coupling between GOT or other transaminases and GPT and since several are widely distributed systems, one must be cautious in accepting alanine synthesis from pyruvate in crude homogenates as unequivocal evidence of direct transamination. Such a coupling seems unlikely in our experiment because there was no chromatographically detectable glutamate in the dialyzed homogenates: α-KG was not estimated. Moreover, amino acids which reacted with α-KG did not always form alanine from pyruvate.

Chromatography of liver and kidney homogenates which had been incubated with aspartate and α-KG showed a ninhydrin positive spot with the R_F of α-alanine in addition to a spot at the R_F of glutamate. Neither spot appeared in the absence of α-KG. If one were α-alanine, it might have been formed by the following reaction:



This is a special example of the coupled reaction discussed above.

The presence of arginase (Hunter, 1929 and Bell, unpublished observations) and an apparent ornithine transaminase in salmon tissues suggests that arginine as such might not transaminate but only *via* ornithine. Attempts to inhibit arginase activity in the presence of 0.02 M arsenite or borate were only partially successful and therefore direct transamination of arginine must remain in doubt.

Asparagine and glutamine appeared to undergo some transamination with α-KG in salmon tissues, and even though direct transamination of the amides has been shown (see Guirard and Snell, 1964), it is possible that in this system they only participate after deamidation.

The overwhelming mass of published data relating to the scope and extent of transamination reactions in various tissues makes it expedient to present a tabular survey of some important data which are relevant to our results and to the discipline of comparative biochemistry (Prosser, 1964) (Table II). Data on aspartate and glutamate are excluded because both GOT

TABLE II. Literature survey of some characterized and uncharacterized enzymatic transaminations relating to the systems in Table I.[a] (P indicates some degree of enzyme purification; +, glutamate or α-alanine formed; 0, no α-alanine formed; N.T., not tested with donor.)

Amino donor	Sources of transaminase(s) (homogenates or extracts)	Amino acceptor		Remarks
		α-KG	pyruvate	
β-Alanine	*Pseudomonas fluorescens* (P) *Periplaneta americana* *Neurospora crassa* (P)	+	+	Not active in pig heart muscle or in the mussel *Mytilus edulis*. In cockroach homogenates donor tested with α-KG only.
γ-Aminobutyrate	Beef brain (P) *Pseudomonas fluorescens* (P)	+	0	Major pathway of metabolism of γ-aminobutyrate in brain (Baxter and Roberts, 1958).
L-Arginine	Pig liver and kidney Locust, cockroach and crayfish	+	N.T.	Methods did not preclude transamination via ornithine. System inactive in crab.
Glycine	Higher plants Locust fat body, crab, and crayfish	+	0	Highly active, widely distributed. Inactive in crab and pig tissues. Pyruvate inactive in rat liver.
L-Histidine	Pig, rat (P), rabbit, cockroach, and locust tissues Crayfish, bacteria	+	+	Weak to no activity in animal tissues with α-KG. Pyruvate acceptor in rat liver. Insects and crayfish tested with α-KG only.
L-Leucine	Chicken liver (P) Rat, pig, locust, crab, and crayfish tissue Plants	+	+	Good activity with α-KG. Pyruvate tested only with rat liver and kidney and crab. Locust tested with α-KG only.
L-Norvaline	*L. arabinosus* *B. subtilis*	+	N.T.	Donor seldom tested.
L-Ornithine	Pig heart, rat liver (P) Locust fat body, crab *Neurospora crassa* (P)	+	+	Weak activity pig heart. Pyruvate better acceptor in rat liver (Quastel and Witty, 1951). δ-NH_2 labile. Locust tested with α-KG only. System inactive in crab.
L-Phenylalanine	Pig, rat, locust tissue Higher and lower plants Crab, crayfish	+	+	Mostly tested with α-KG: good activity. Might be concentrated in particulate fraction of rat kidney. System inactive in crab.

L-Proline	Cockroach Crayfish	+	N.T.	Donor seldom tested because it is an imino acid but possible it might act after oxidative rupture of pyrrolidine ring. Proline not true substrate. Inactive in locust.
L-Serine	Dog liver (P) Pig heart *Mytilus edulis* Locust, crayfish	+	+	Weak or doubtful activity in pig heart and *Mytilus edulis* (only α-KG tested). Donor and α-KG inactive in cockroach and crayfish. No activity with pyruvate in rat liver.
L-Threonine	Pig heart, liver, and kidney Locust, cockroach, crayfish *P. fluorescens, E. coli*	+	N.T.	Weak or no activity in pig tissue and *M. edulis*. Moderately active in the bacteria.
L-Tyrosine	Rat (P), pig, and dog (P) organs Locust, crayfish Various bacteria: yeast	+	N.T.	Most active in liver and kidney. Rat transaminase (P) also acts on phenylalanine and tryptophan (Jacoby and La Du, 1964). Enzyme induced in vivo and in tissue culture by hydrocortisone (Tomkins et al., 1966). Inactive in cockroach. Active with α-KG but not pyruvate in crab.
L-Valine	*E. coli* (P), locust, cockroach and mammalian tissues, higher and lower plants Crab and crayfish	+	+	Transaminase using α-KG appears to be widely distributed. As usual α-KG most frequently tested NH_2 acceptor. No activity with pyruvate in rat liver or crab tissues. Pyruvate not tested with crayfish.

[a]Adapted principally from reviews by Cohen and Sallach (1961) and Guirard and Snell (1964). Additional data from Cammarata and Cohen (1950) on pig tissue; Rowsell (1956a, b) on rat; Kilby and Neville (1957) on locust (*Schistocerca gregaria*); McAllan and Chefurka (1961b) on cockroach (*Periplaneta americana*); Read (1962) on mussel (*Mytilus edulis*); Chaplin et al. (1967) on crab (*Carcinus maenas*); and Urich (1967) on crayfish (*Orconectes limosus*).

and GPT are discussed at length in the text. The usual pattern of transamination in tissues indicates that GOT and GPT are the most active and widely distributed, although GPT could not be detected in the blood of certain invertebrates (Zandee et al., 1958; Price, 1961) and GOT could not be detected in the hemolymph of the roach (McAllan and Chefurka, 1961a) or in the blood of *Mytilus edulis* (Read, 1962). Read's data from whole, undialyzed tissue homogenates and a test series of amino acids similar to those in Table II suggest that GOT and GPT are the only active transaminases in *Mytilus*.

Effect of Hepatic Poisons on SGOT Levels

Preliminary analysis of the data in Table III indicated that SGOT levels were not correlated with weight, length, or sex and further analysis showed that there was a highly significant difference ($p < 0.001$) between the SGOT levels of control and treated fish. Unfortunately we did not verify histologically that hepatic damage had occurred as a result of poisoning and so the mechanism causing elevated SGOT levels is open to speculation.

Table III. Effect of intraperitoneally injected bromobenzene and CCl_4 on SGOT levels in juvenile sockeye salmon.[a,b,c]

Weight (*g*)		Length (*cm*)		Sex		SGOT (*units*)	
Control	Treated	Control	Treated	Control	Treated	Control	Treated
203	178	27	26	♂	♂	250	900
200	154	27	23	♂	♀	400	1050
120	186	24	26	♂	♀	320	950
132	124	24	22	♀	♀	250	1600
117	226	23	29	♀	♂	400	900
100	138	22	22	♀	♂	200	1300
185	154	26	25	♀	♀	270	1250

[a]For data on each control and treated fish read across.
[b]Injected with a 1:1 mixture of bromobenzene and CCl_4 at a dosage of 450 mg bromobenzene/kg body weight. Controls injected with similar volumes of saline.
[c]Sera obtained at 15 hr because fish became moribund.

Molander et al. (1955) caused hepatocellular damage by feeding rats CCl_4 and found that SGOT was a very sensitive indicator of tissue destruction. But Wróblewski and La Due (1956) found SGPT to be an even more sensitive indicator of acute hepatocellular damage. The ratio of the transaminase levels depends upon the type of liver disease, as pointed out in the review by Agress (1959).

Effect of Kidney Disease on SGOT Levels

The data presented in Table IV were subjected to the *t*-test after preliminary analysis indicated that there was no need to adjust for differences in weight or length of healthy and diseased populations when comparing the

TABLE IV. Hematocrits and PGOT levels in healthy and kidney diseased male and female *O. nerka* of various weights and lengths.[a,b]

Weight (g)		Length (*cm*)		Sex		Hematocrit		PGOT (*units*)	
Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
58	22	17	14	♂	♂	36	19	600	1400
24	40	14	17	♂	♀	31	33	250	380
24	29	14	14	♀	♂	25	20	350	900
60	25	19	14	♂	♀	43	14	850	1020
43	36	17	16	♂	♂	32	11	500	1100
41	19	17	14	♂	♂	30	18	300	1250
46	28	17	14	♂	♂	38	12	260	360
45	42	17	17	♀	♂	34	29	400	330
51	21	18	14	♂	♀	31	13	360	1100
74	29	19	16	♂	♀	34	17	400	1200
65	21	18	14	♂	♂	35	29	330	1430
63	25	19	15	♀	♀	32	34	350	400
53	39	18	16	♂	♂	31	34	380	250
52	58	18	19	♂	♀	34	40	420	330
41	35	17	16	♂	♀	31	37	350	270
33	21	16	14	♂	♂	38	27	580	520
56	58	18	18	♂	♂	41	31	350	360
33	41	15	16	♂	♀	31	19	320	355
81	42	20	17	♀	♀	44	30	530	1400
31	46	16	17	♀	♀	44	15	320	1200
38	45	16	17	♂	♀	36	5	280	900
44	53	17	18	♀	♀	17	14	350	1200

[a]For data on each healthy or diseased fish read across.
[b]Condition of health or disease determined by macroscopic examination for lesions in internal organs. Severity of disease not considered.

means of hematocrit and PGOT values of these populations. Table V summarizes the results of these analyses and it can be seen that there were significant differences between healthy and diseased fish (males and females) based on measurements of weight, length, hematocrit, and PGOT. The most consistently significant differences were obtained using the PGOT test. Since there were no data on weights and lengths before the culture of fish became

TABLE V. Probabilities associated with calculated *t* values for different criteria used to distinguish between healthy and diseased *O. nerka* (see Table IV).

Grouping	Weight	Length	Hematocrit	PGOT
Male	0.004(24)[a]	0.002(24)	<0.001(24)	0.006(24)
Female	0.221(16)	0.348(16)	0.090(16)	0.029(16)
Combined sexes	0.004 (42)	0.006(42)	<0.001(42)	<0.001(42)

[a]Degrees of freedom in parentheses.

infected and, moreover, because it was unlikely that there was a synchronous infection, the measured differences in weights and lengths cannot be unequivocally ascribed to disease. In any case, weights and lengths as diagnostic measures would likely be useful only under very limited conditions.

More useful assessment of the diagnostic sensitivity of the various tests might have been obtained if the diseased population had been graded on some arbitrary measure of disease severity.

Distribution of GOT in Tissues

The data in Table VI, in addition to giving examples of total N in various tissues which have been used in this work, suggest that the distribution of GOT in salmon generally parallels that in the dog and is of the same order of magnitude (Dubach, 1957). (Precise comparison is not possible because Dubach used a colorimetric assay for GOT.) The exception to this parallelism is that skeletal muscle of the dog is more active than kidney whereas the reverse appears to be true in salmon. Until more complete results have been obtained and analytical considerations such as those described by Thiers and Vallee (1958) have been thoroughly explored, the apparent order of specific activities of GOT in salmon, viz., heart > liver > kidney > muscle must be regarded as tentative.

The rationale for using SGOT or other serum enzymes and isoenzymes for clinical diagnosis was that elevated levels should simply reflect the amount of damage which had occurred in enzyme-rich tissues and also, therefore, that the ratios of enzyme activities in serum and affected tissue should be similar. White (1958), however, was unable to detect any clear relationship between the abnormal serum enzyme level and the level of the respective enzyme in tissue homogenates. Obviously, other factors are involved in the production of such abnormalities in humans (Bodansky, 1958) and probably also in salmon but assay of serum enzymes remains empirically useful even if rationally vague.

DISCUSSION

Our preliminary results using partially purified GOT from salmon liver suggest that the enzyme(s) might resemble GOT obtained from other tissues, but valid comparison must await the characterization of the salmon enzyme or isoenzymes. Salmon apo-GOT, like its counterparts from other organisms, is activated by PLP, thus demonstrating a physiological function for vitamin B_6, a dietary requirement for salmon (Halver, 1957). Salmon holo-GOT behaves like the pig heart enzyme in that it could best be resolved by precipitation from acidic solutions with ammonium sulfate (Wada and Snell, 1962). In a preliminary communication Johnson (1967) reported a substantial lowering of GPT activity in the erythrocytes and livers of thyroidectomized trout. It would be interesting to know which component of the holoenzyme was affected by the thyroidectomy because active apotransaminase synthesis can

TABLE VI. Total nitrogen and GOT estimations of various tissues of *O. nerka* and *O. kisutch*.

				mg N/g wet weight tissue				Units GOT/mg N ($\times 10^3$)			
Fish	Species	Weight (g)	Sex	Liver	Kidney	Heart	Muscle	Liver	Kidney	Heart	Muscle
1	Sockeye	67	♂	18.2	14.5			5.7	4.8		
2		85	♀	18.8	15.4			4.6	3.6		
3		97	♂	18.0	16.4	11.5		5.0	4.3	7.9	
4	Coho	168	♀	20.9		16.2		4.6			
5		172	♂	21.4	19.4	14.6		6.9	2.4		
6		260	♀	21.7	17.9	16.6	28.7	5.0	3.1	6.1	0.5
7		309	♂	21.4		16.8[a]	27	5.3		6.8	1.1
8		270	♀	19.6	18.7		28.4		3.2		0.7

[a]One value only: all other estimations are average of duplicates.

occur in cofactor-deficient cells (see Guirard and Snell, 1964). It is therefore often advisable to supplement test systems with PLP. (We have found active SGOT in ripe, adult sockeye (Bell, unpublished results), thus giving indirect support to the observation of Cowey et al,. 1962, that certain B vitamins are retained in the tissues of adult, nonfeeding salmon).

The scope of transamination reactions in salmon tissues is probably much less limited than indicated by our results. The trend of research has been to discover more and more transaminase reactions with the use of more sensitive methods such as employing concentrated, dialyzed tissue extracts supplemented with PLP (Cammarata and Cohen, 1950; Jacoby and La Du, 1964). The failure of some workers (Rowsell, 1956a and b; Read, 1962; Chaplin et al., 1967; and Urich, 1967) to supplement tissue homogenates with PLP could have imposed false limitations on their results. It is now considered somewhat remarkable if a naturally occurring amino acid is definitely found not to transaminate. It seems reasonable, therefore, to suggest that amino acids which showed weak or doubtful degrees of transamination in salmon tissues are actually positive. Further, weak transamination need not imply physiological insignificance (Meister, 1955).

"Studies with unwashed whole homogenates can give useful information on the overall amino transfers possible in tissues but leave doubt as to the individual transamination steps involved" (Rowsell, 1956a). In our exploratory study, mechanisms were not our concern, only amino transfer, but it seems that apparent transamination in salmon tissues could account for the biosynthesis or transformation of a number of amino and keto acids. Moreover, transamination to α-KG, coupled with the action of glutamic dehydrogenase (L-glutamate: NAD or NADP oxidoreductase (deaminating)), in other words "transdeamination," might be an important mechanism of ammonia formation in salmon as in other fishes (McBean et al., 1966) and mammals (Miller et al., 1955). Lack of functional integration, however, might prevent such a mechanism from operating in intact tissue even if the individual reactions could be demonstrated in vitro. Rowsell (1956b) cites an example in which brain slices were unable to oxidize L-aspartate even though brain contains active GOT and L-glutamate oxidizing systems.

Serum transaminases and other enzymes in the blood have been widely used for clinical diagnosis in humans (Whitelock et al., 1958; Agress, 1959; and Ruyssen, 1962). However, without supplementary tests and observations coupled with knowledge of "normal" (Bodansky, 1958 and Copeland, 1962) or "standard" (for fish cultured under standardized conditions) values for the species, subpopulation or, ideally, for the individual (Bodansky, 1958; Prosser, 1964), clinical enzymology like other diagnostic tools would usually be of little value. It should therefore become a widely supported goal of fisheries research to produce tables of normal ranges of physiologic parameters such as exist for humans. In our experiments kidney disease could not have been diagnosed by the SGOT test alone because, like Hunn (1964), we have only found a correlation between this particular disease and the level of a certain

body constituent. This correlation is sufficient to distinguish healthy from diseased fish with a high degree of certainty but the degree of specificity and sensitivity of the GOT and GPT tests have yet to be determined in fish.

Although SGOT levels are claimed to be remarkably constant in humans under various circumstances (Agress, 1959), elevations have been reported after strenuous exercise in man, monkey (Robinson et al., 1964), and rat (Altland and Highman, 1961). Subcutaneous injection of epinephrine in oil also caused elevation in levels of both SGOT and SGPT in several warm-blooded animals including the rat (Garbus et al., 1967). In contrast, Rasmussen and Rasmussen (1967) found that SGOT remained constant in fatigued sharks whereas serum lactic acid dehydrogenase increased 1.5-2 times. (Tissue GOT but not GPT remained constant (based on wet weight tissue) in aestivating African lungfish (Janssens, 1964).) The stability of SGOT levels in salmon was not investigated but any effects of our sampling procedures should have tended to be random.

The reason for the elevation of P or SGOT levels in poisoned or diseased salmon is open to conjecture but the following are some possibilities: (*a*) leakage of enzyme from damaged tissue, erythrocytes or bacteria; (*b*) increased synthesis of enzyme by a tissue; (*c*) impaired mechanism for removal of serum enzyme; (*d*) hemoconcentration, giving an apparent increase in GOT; (*e*) hemolysis of erythrocytes by bacterial hemolysins or exogenous poison.

Fish culturists need an array of simple, reliable, sensitive tests of various degrees of specificity which can be used to monitor stocks for incipient disease — whether physiologic or infectious — without having to kill the subjects. The hematocrit so successfully introduced into fishery management by Snieszko (1960) is one such test and there is no a priori reason to suppose that clinical medicine will not offer further tests which can be used for the surveillance of cultured fish at a general or specific diagnostic level. Our results further confirm the value of hematocrits as a means of judging state of health or separating diseased from healthy specimens. The simplicity of the test is also much in its favour.

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Starch in the Oceans[1]

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ABSTRACT

Starch observed in arctic waters of Canada, in the Baltic and Mediterranean seas, in the North Atlantic and Indian oceans, and in the waters about Bermuda, the Virgin Islands, and Jamaica is formed mostly in Gymnodinioideae, benthic algae, and higher aquatic plants and rarely in planktonic Chlorophyceae. In these phytoflagellates the fragile pellicle breaks when there are critical changes in salinity, temperature, or pressure and is subsequently decomposed by bacteria. The starch retains its shape after destruction of the protoplast, and accumulates in large quantities in aquatic habitats. Standing crops of free starch grains mark past blooms and mass mortalities of phytoplankton. The largest potato-like starch grains found in the oceans appear to be derived from the ocellar lenses of Warnoviaceae.

Though Protozoa and various herbivores may ingest free planktonic starch, they were not observed to digest it. Large standing crops of starch and monosaccharides derived from amylolysis may affect nutrition in some animals, and influence the optical and chemical properties of sea water. Amylogenesis in some dinoflagellates starts from chondriosomes which are specialized in this process. In Prorocentrideae starch is formed within the ectoplasmic reticulum.

INTRODUCTION

My first observations on starch in suspension in sea water were made in 1934 from the Polish Marine Station at Hel on the Baltic coast. Starch-like bodies were noticed again in the Mediterranean plankton (Bursa, 1959). Similar structures occurring in the coastal waters of Point Barrow, Alaska (Bursa, 1963) and in Hudson Bay and Foxe Basin (Bursa, 1961a, b) appeared to originate from unidentified phytoflagellates.

This is a report on intensive quantitative and morphogenetic studies on over 2000 formalin-preserved samples of phytoplankton from pelagic and coastal waters of the Canadian Archipelago and the Arctic and North Atlantic oceans. Though no starch producers were identified with certainty, a few intact Gymnodinioideae permitted identification of starch grains found extracellularly by comparison with the forms found in situ. Starch of tropical waters is also described, and contrasted with the arctic type. Observations on amylogenesis in living dinoflagellates are discussed since they are relevant to the general problem.

Amylogenesis

Starch grains in Phytoprotista develop from the chondriosomes. Presumably, as in *Woloszynskia limnetica*, some of the chondriosomes are specialized in amylogenesis. Though advanced phases of the grains can be studied

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in vitro, their early morphodynamics should be studied in living protoplasts.

Chondriosomes in *Exuviella mariae-lebourae* stain with Janus green (Parke and Ballantine, 1957). The same pigment gave faint greenish colouration of the chondriosomes in *E. cassubica* Woloszynskia and other Prorocentrideae whose chondriom consists of both nonamylogenic and amylogenic units. The staining capacity of amylogenic chondriosomes decreases with the accumulation of starch molecules around them.

In Prorocentrideae amylogenesis takes place within the ectoplasmic reticulum, which may be isolated for study from the living cell (Fig. 2). In the genus *Exuviella* two patterns of starch formation were found. In *E. cassubica*, ectoplasmic reticulum (Fig. 1, 2) contains spherical chondriosomes at the junctions of all the tubular meshes, but starch is catalysed only within those chondriosomes located close to the membrane border (Fig. 1). In an undescribed species of *Exuviella* (Adam S. Bursa, unpublished information) and other species of the genus, pyrenoid-like structures (Fig. 2, 3) are found.

The division of amylogenic chondriosomes could be inhibited by excessive accumulation of starch molecules around them. Aberrant starch grains (Fig. 1, *asl* and *asll*) may then be formed.

The amylogenic centres usually dissolve in the early stages of development of the starch grains (Fig. 1, *rs*; Fig. 2, arrow 2) but often remain until the later stages (Fig. 9, 11). The centres may be destroyed also by mechanical fracturing (Fig. 33, 37, 39, 42, 46), both in living protoplast and in extracellular grains. However, destruction of the centre in living protoplasm does not inhibit the growth of the starch grains. In extracellular starch, loss of the centre is accompanied by swelling, gelatinization, and hydrolysis. In starch grains, or the lenses, of some Warnoviaceae (Fig. 57–61) the centres evidently atrophy at an early stage of development.

As in *W. limetica*, a blue–red pigment, possibly antocyan, seems to determine the course of development of the grains. In the Warnoviaceae, the pigment seems to exist both in the melanosomes of ocelli and in the chondriosomes from which the lenses (Fig. 9, 11) generate.

The above account supports my suggestion that amylogenic chondriosomes act as autodynamic organs, not entirely controlled by the nucleus. This view is also supported by experiments on enucleated specimens of the calcareous alga *Acetabularia* (Schweiger et al., 1964; Brachet, 1965).

METHODS

A microscope with phase-contrast equipment was used for general observations and photomicrography in the sedimentation chambers. Only plankton net hauls taken with a No. 20 net were used for the morphological observations.

The starch in phytoplankton samples was often so gelatinized that it disintegrated under cover-glasses (No. 0) while the compound microscope preparations were being made. It always settled on the bottom of the sed-

imentation cylinders and thus permitted both morphological and quantitative observations, according to methods previously used (Bursa, 1961a, b).

The characteristics of different starch bodies found in situ in plankton organisms, and free in sea water, were determined by birefringence and the blue starch–iodide reaction. The calcareous coccoliths of *Scyphosphaera* sp(p). (station 21) also revealed birefringence caused by their layered structure (Fig. 38). Exceptionally hydrolysed grains treated with iodine turned a reddish colour, indicating the presence of dextrine. Vital staining of Gymnodinioideae was effected with cresyl blue, neutral red, and Janus green dissolved in sea water, in concentrations of 0.3, 0.5, and occasionally 1.0%. The ectoplasmic reticulum and amylogenic chondriosomes from living *Exuviella cassubica* were extracted by squeezing the dinoflagellates between thin mica plates with tungsten needles.

OCCURRENCE, STRUCTURE, AND PRODUCERS OF STARCH

Starch grains were found in arctic waters of Canada, in the North Atlantic Ocean, and various other waters (Table I). The abundance varied widely, the amounts per cubic metre at three arctic stations (Table II) being as high as 100–3155 mg. At some stations no starch grains were found either extracellularly or in living protoplasm.

Starch grains produced by holophytic Gymnodinioideae were observed to belong to two morphological groups. In *Gymnodinium*, *Gyrodinium* (Fig. 15), *Woloszynskia*, *Amphidinium*, and *Massartia*, the grains are irregularly spherical, discoid, cubical, cylindrical, polygonal, or rod-shaped. In the Warnoviaceae, the grains are mainly spherical, as in reserve starch, or mainly lens-shaped (Fig. 16, 17), as in functional forms in ocelli.

Some of the dinoflagellates examined produced characteristic forms of starch grains. In the Warnoviaceae (Fig. 4–17, 41, 43, 44) the ocellar lenses evidently differed according to species. The structure was shown in greatest detail when the lenses had disintegrated during vital staining (Fig. 14, 16, 17). Lens-like grains taken from the drifting ice island T-3 at pelagic stations in the Arctic Ocean had shallow surface depressions (Fig. 57, 58, 60, 61). In other families, characteristic differences were found as follows: discoid and spherical grains in *Ceratium* sp. (Fig. 29), polygonal grains in some species of *Gyrodinium* (Fig. 15), twin grains in *Pyraminos* sp. (Fig. 27, 28), and deltate grains in *Polyblepharis* (Fig. 30).

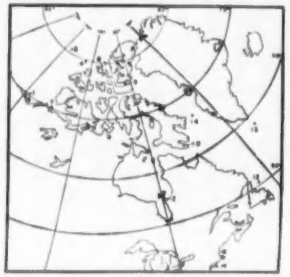
Of extracellular starch grains found in pelagic waters (Fig. 36, 37, 39, 44–50, 56), some appeared to be lenses of Warnoviaceae. A few intact formalin-preserved cells of *Pronoctiluca pelagica* (Fig. 41) and *Cochlodinium* sp. (Fig. 43) permitted identification of extracellular grains (Fig. 36 and 44, respectively) by comparison with grains in situ.

In lens-like starch, the smaller grains were found to consist of 30 or fewer layers (Fig. 57), and the largest of more than 80 (Fig. 58–61).

Many characteristic small forms of starch grains occur in arctic littoral waters (Fig. 27–30). They appear to be formed in Chlorophyceae and some

TABLE I. Arctic and tropical plankton stations and collections referred to in text (*see map*: tropical stations not shown).

No. of collection	Date	Locality	Coordinates	Collector	Method of sampling and size of net
(1)	21–8–51	Beaufort Sea	70°04′N 138°06′W	*Cancolim II* expedition	Vertical net tow, No. 6
(2)	1–9–58	Belcher Is.	55°15′N 79°30′W	*Calanus* expedition, E. H. Grainger	Quantitative phytoplankton samples
(3)	6–9–59	Belcher Is.	55°55′N 79°39′W	*Calanus* expedition, E. H. Grainger	"
(4)	8–7–58	Arctic Ocean, Fletcher's Ice Is. (T3)	79°47′N 116°22.5′W	Arctic Institute of North America expedition, S. Apollonio	"
(5)	8–8–64	Arctic Ocean, Fletcher's Ice Is. (T3)	80°29.5′N 134°04′W	M. Weinstein, G. Harding	"
(6)	6–8–60	Herschel Is., Beaufort Sea	69°38′N 138°35′W	*Salvelinus* expedition, J. G. Hunter	Horizontal net tow, No. 25
(7)	1–7–64	Cape Parry, Beaufort Sea	70°08.9′N 124°39.9′W	*Salvelinus* expedition, I. G. Gidney	Diatom film taken from ice subsurface
(8)	6–6–60	Ellef Ringnes Is.	78°45′N 103°30′W	Canadian Polar Shelf expedition, A. S. Bursa	Mud taken with Ekman sampler; quantitative phytoplankton samples
(9)	7–6–63	Devon Is.	75°40′N 84°33′W	Arctic Institute of North America expedition, S. Apollonio	Quantitative phytoplankton samples (for collections (9) to (14) inclusive)
(10)	14–7–62	Ogac Lake, Baffin Is.	62°52′N 67°21′W	I. A. McLaren	
(11)	27–8–62	Cambridge Bay, Victoria Is.	69°10′N 106°20′W	L. Johnson	
(12)	6–9–61	Northwest Atlantic	50°19.8′N 54°04.3′N	*Sackville* expedition, ICNAF	
(13)	29–5–63	Labrador Sea	58°30′N 43°00′W	*Sackville* expedition ICNAF	
(14)	10–6–63	Davis Strait	66°33.5′N 56°38′W	Baffin expedition ICNAF	



(15)	6–1–61	Bermuda Biological Station	32°20′N 65°45′W	A. S. Bursa	Horizontal net tow, No. 25
(16)	24–8–64	Tortola, British West Indies	18°34′N 64°40′W	"	"
(17)	28–3–65	Key West, Florida	24°09′N 82°05′W	"	"
(18)	2–9–52	Eel Pond, Woods Hole, Mass.	41°31′N 70°42′W	L. Provasoli	Culture (clones of *E. cassubica*)
(19)	18–8–58	Long Island, N.Y.	40°50′N 72°50′W	R. R. Guillard	Culture (clones of *E. intermedia*)
(20)	13–9–65	Ocho Rios, Jamaica	18°N 77°W	A. S. Bursa	Diatom crust from sand surface

TABLE II. Extracellular starch crops at some arctic stations.

Belcher Islands (station 2), September 11, 1958									
Depth (*m*)	0	10	25	50					
No. of grains per litre (*thousands*)	45	107	250	100					
Total wt (*mg in 4 litres*)	0.4								
Mg/m³	100.0								
Belcher Islands (station 3), September 6, 1959									
Depth (*m*)	0	1.5	3	5	10				
No. of grains per litre (*thousands*)	190	164	250	106	100				
Total wt (*mg in 5 litres*)	0.6								
Mg/m³	120.0								
Cambridge Bay (station 11), August 27, 1962									
Depth (*m*)	0	3	5	7	10	15	20	30	49
No. of grains per litre (*thousands*)	1906	3368	963	324	6592	3219	4183	7592	389
Total wt (*mg in 9 litres*)	28.49								
Mg/m³	3155.5								

Figures 1–14

FIG. 1. *Exuviella cassubica* in valvar view (station 19): *f*, flagellum; *pc*, discharged content of pusule; *ep*, ectoplasmic reticulum of the pusule; *tr*, discharged trichit; *he*, hyaline ectoplasm discharged through membrane pores; *mp*, membrane pores; *pr*, pyrenoid penetrated by the ectoplasmic reticulum containing chondriosomes; *rs*, regular starch grains; *asl*, aberrant starch (two long trichits are indicated by arrow heads); *asll*, long aberrant starch. Camera lucida, 2500×.

FIG. 2. Ectoplasmic reticulum dissected from living *E. cassubica*. Arrow 1: nonamylogenic chondriosomes within the tubules; arrow 2: amylogenic chondriosomes at the moment of destruction by cavitation. Squares contain nonamylogenic chondriosomes dividing in the early stage of their lives. Camera lucida, 2500×.

FIG. 3. Ectoplasmic reticulum of an underscribed species of *Exuviella* containing a spherical starch grain surrounded by rodlets of starch, in vivo. Camera lucida, 2500×.

FIG. 4–14. Ocelli of arctic Warnoviaceae, discharged from living cells, consisting of dark melanosome pigment and having the base of each lens hidden. *Fig. 4*. Constricted lens characteristic of *Cochlodinium* sp. (station 2). *Fig. 5*. Central constriction in the lens of *Warnovia* sp., suggesting tendency towards division and formation of twin lenses (station 2). *Fig. 6*. Starch-like layers in the lens of *Erythropsis* sp. emerging from the melanosome (station 12). *Fig. 7*. Non-layered lens of *Cochlodinium* sp. (station 2). *Fig. 8*. Irregular form of lens of *Cochlodinium* sp. (station 8). *Fig. 9*. Starch-like layers observed in *Cochlodinium* sp. (arrow indicates the blue–red amylogenic chondriosome). *Fig. 10*. Characteristic lens of *Cochlodinium* sp. (station 2). *Fig. 11*. Lenses in some genera of Warnoviaceae originate from two amylogenic chondriosomes, as shown by arrow (station 2). *Fig. 12*. A bizarre form of ocellus characteristic of *Cochlodinium* sp. (station 2). *Fig. 13*. Lens of *Pouchetia* sp. consisting of many transverse units (station 2). *Fig. 14*. Structure of lens of *Cochlodinium* sp. shown while lens was disintegrating during the vital staining process (station 2). Free-hand drawing. Figures 4–13, camera lucida, 500×.

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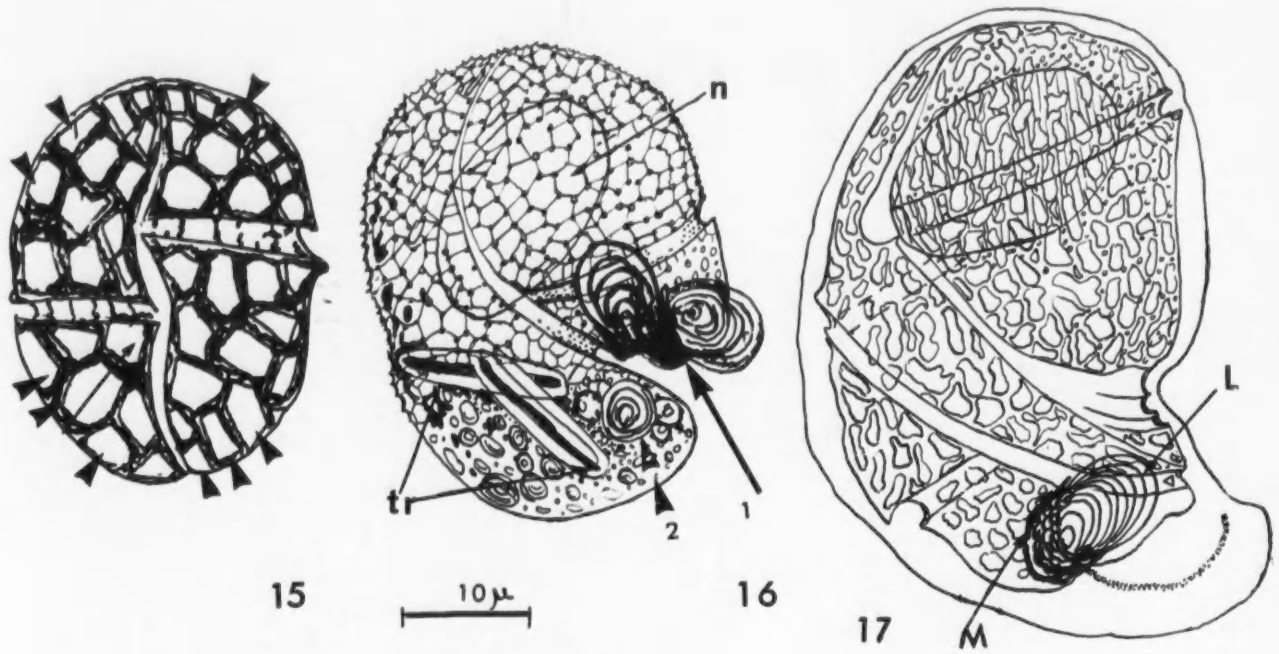


Figures 15–17

FIG. 15. *Gyrodinium* sp. observed in vivo, containing polygonal grains developed from spherical grains found in juvenile cells (station 2). Camera lucida, 500×.

FIG. 16. Living *Cochlodinium* sp. containing twin lenses (station 2). The protruding lens (arrow 1) may have been extruded by a very low concentration of brilliant cresyl blue. Large arrowheads indicate reserve starch formed in the protoplasm or chromatophores. Camera lucida, 500×.

FIG. 17. Encysted *Cochlodinium* sp. showing lens (L) and melanosome (M) of ocellus (station 2). In each of the two *Cochlodinium* species (Fig. 16 and 17) the lenses reveal two distinctive structures appearing during disintegration caused by vital staining. Camera lucida, 500×.

may be identified by comparison with drawings from Butcher (1959). This kind of free starch is never abundant in either tropical or arctic waters. Starch from the benthic algae Florideae (Fig. 51–53) is rare and was observed in only a few inshore stations. All forms of starch found in the inshore tropical waters of Bermuda (station 15), Tortola (station 16), the Florida Keys (station 17), and Jamaica (station 21) differ from arctic types in shape and layering of the grains (Fig. 35, 45, 49, 50). The starch shown in Fig. 54 and 55 appears to have been formed in cells of higher plants that were later macerated.

DISCUSSION

Liberation of Starch

Necrocenoses (mass mortalities) of Gymnodinioideae, whose fragile pellicles disintegrate during ice melting and under exposure to critical changes in salinity, temperature, or hydrostatic pressure, were observed often by Bursa (1961a, b). Many Chlorophyta and Gymnodinioideae in the diatom film on the ice subsurface were observed to be destroyed by similar factors, but more often by bacteria and fungi.

The mass mortalities of the marine Phytoprotista appear to be accompanied by starch liberation. The greatest standing crops of starch were observed to occur simultaneously with the late summer maxima of dinoflagellates (Bursa, 1961a, b) in the Canadian arctic. At the same time faecal pellets containing starch, which evidently resulted from the peak of grazing activity of zooplankton (Grainger, 1959), were found in many areas. Mass mortalities of autotrophic Gymnodinioideae were observed during the fall in inshore Baffin Island waters.

Factors which induce phytoplankton necrocenoses were elucidated by observations of Mr Spencer Apollonio (personal communication) made during the Arctic Institute of North America's Devon Island Expedition in 1960–63. According to his observations, long periods of sunny weather stimulate the rapid increase of phytoplankton in the surface waters, huge populations of which deplete all nutrients. My quantitative and qualitative analyses of 3 years' standing crops observed during the expedition invariably showed that almost complete exhaustion of nutrients in highly stratified waters leads to the starvation of bacterioplankton and marine fungi, which then attack phytoplankton and often zooplankton, destroying it en masse. Not only diatoms but also Gymnodinioideae are destroyed by bacteria.

Biological Significance of Free Starch

Some of the unpreserved samples of water from the Belcher Islands (station 3) were moderately opalescent when observed against the light. The formalin-preserved plankton samples containing many millions of starch grains (Table II) revealed more intensive opalescence and turned blue when a few drops of iodine were added to the vial. There seems little doubt that the

FIG. 17. Encysted *Cochlodinium* sp. showing lens (L) and melanosome (M) of ocellus (station 2). In each of the two *Cochlodinium* species (Fig. 16 and 17) the lenses reveal two distinctive structures appearing during disintegration caused by vital staining. Camera lucida, 500×.

starch contributes extensively to the scattering of light which takes place in the surface layers of sea water.

Since the amount of starch in suspension and its disassociated fractions must be fairly high, judging from the number of grains found per litre of sea water (Table II), the bacterial flora and fungi which metabolize carbohydrates (Kriss, 1963) are likely to be invigorated to some extent. Though it is not known whether the Protista and other grazers utilize free starch and its dissolved products in aquatic habitats, it is certain that all these substances increase the concentration of arctic leptopel (Goldberg et al., 1952).

Since caloric and nutritional values for planktonic starch are not yet available, an approximate value was obtained by contrasting the numbers of grains of starch per litre in the standing crop of plankton with the number of grains per milligram in potato starch. Such a comparison is instructive within limits, since planktonic and potato starch grains consist of similar size-groups. In my terminology small grains vary from 0.5 to 10 μ, medium-size grains from 10 to about 23 μ, and the largest grains from 23 to 36 μ. By maceration and examination of potato starch in a 10-ml sedimentation chamber, 1 mg was found to contain an estimated 250,520 grains: 154,700 small, 58,080 medium, and 37,740 large. According to De Willigen (reported by Whistler, 1964), a potato about 4 $\times$ 2 inches contains 16% or more starch, and 1 kg should yield from 80 to 100 g of dry starch. As indicated in Table II, a litre of sea water was found to contain as many as 9 million grains.

It is likely also that dissolved starch derivatives change the osmotic properties of water itself, influencing metabolism of both facultative and nonfacultative osmotrophic organisms in both phytoplankton and Zooprotista.

Importance of Starch as Animal Food

The content of carbohydrates in *Chlorella* spp. varies from 6 to 57% of dry weight, depending on nutrients, according to Strickland (1960). Though no planktonic organism studied in a living or preserved state was found to digest free starch, the observations in this paper indicate the occurrence of a hitherto unknown and abundant source of food. Gymnodinioideae are known to ingest diatoms, phytoflagellates, and small dinoflagellates (Schiller, 1933; Biecheler, 1952; Bursa, 1961a, b). The holozoic species *Gyrodinium spirale* and *Amphidinium semilunatum* (Fig. 32, 34), with their extensible pellicle, were observed ingesting many cells of Peridineae, Chlorophyceae, and even small Warnoviaceae. Most organic compounds ingested by these organisms appeared to be enzymatically digested and absorbed into the protoplasts, but starch and cellulose usually remained intact (Fig. 32, 34) and were eventually excreted. The voracious species *Discoasteromonas calciferus* (Bursa, 1965) was observed ingesting at one time as many as eight small flagellates such as *Pyramimonas*, *Polyblepharis*, and *Chlorella* (Fig. 32), and also small pennate diatoms. Though cellulose membranes and silicaceous frustules of

Explanation of Figures

Figures 18–34

FIG. 18. Gelatinized starch and particles of organic detritus, settled on the surface of a desimentation chamber (station 11). 400×.

FIG. 19, 20. Amylolysis in some extracellular starch grains begins at a single point near an edge. 400×.

FIG. 21. Amylolysis in another kind of grain begins from a number of peripheral dark spots, shown by arrows (station 9). 400×.

FIG. 22. Arrow indicates amylogenic centre, replaced by starch molecules. This form of starch occurs in many arctic habitats (stations 1–9). 400×.

FIG. 23. Faecal pellet excreted by an unidentified gymnodiniophage, containing undigested starch (station 11). 100×.

FIG. 24. A polygonal starch grain upon *Navicula* sp. (station 10). 400×.

FIG. 25, 26. Reticular structure, more highly resistant to hydrolysis than the interstitial substance (station 11) 400×.

FIG. 27, 28. Twin antapical starch grains from *Pyramimonas* sp., Chlorophyceae (station 7, 9). 400×.

FIG. 29. Starch of *Ceratium* sp. in polarized light (station 13). 400×.

FIG. 30. Antapical starch from *Polyblepharis* sp., Chlorophyceae (station 3). 400×.

FIG. 31. Discoid starch of unidentified plant showing radial rays and central star-shaped structure, important in identification of the plants producing it (station 13). 400×.

FIG. 32. *Discoasteromonas calciferus* containing intact starch of dinoflagellates (arrows) (station 9). 250×.

FIG. 33. Polygonal grain of planktonic starch formed possibly by the gymnodinioid shown in Fig. 15 (station 14). 400×.

FIG. 34. Living *Amphidinium semilunatum* (Sandy Cove, Nova Scotia). Short arrow indicates a large starch grain, ingested through the sulcus. Long arrows indicate nucleus. 400×.

Figures 35–53

FIG. 35. Starch from Bermuda plankton (station 15). 200×.

FIG. 36. Free starch of *Pronoctiluca pelagica*, staining blue with iodine in preserved state though not in vivo (see Schiller, 1933). 200×.

FIG. 37. Lens-like grain, presumably of *Cochlodinium* sp., appears to have spiral arrangement of layers, shown by arrows (station 13). 200×.

FIG. 38. Birefringent coccolith of *Scyphosphaera* sp. in polarized light (station 22). 200×.

FIG. 39. Lens-like grain, presumably of *Cochlodinium* sp., showing radial striae and characteristic layers (station 13). 200×.

FIG. 40. A semicompound starch of unknown origin (station 6). 200×.

FIG. 41. Intact *P. pelagica*, preserved in formalin (station 5, T-3); starch-like body identical with extracellular starch shown in Fig. 36. The starch varies in size and shape from spherical grains to grains like those of potato starch. 250×.

FIG. 42. Crushed lens-like grain, presumably of *Cochlodinium* sp., showing spiral organization of layers (station 12). 1000×.

FIG. 43. *Cochlodinium* sp., partially deformed by preservation, containing starch grains (station 10). 200×.

FIG. 44. A clump of starch of *Cochlodinium* sp. deposited in the plankton after disintegration of the pellicle (station 10). 200×.

FIG. 45. Starch from Tortola (station 16). Arrow shows missing amylogenic centre. 400×.

FIG. 46. An unidentified starch showing regular layers and birefringence in polarized light (station 12). 400×.

FIG. 47. An unidentified starch grain (station 4). From the centre to the antapex the grain consists of gradually widening, very dense layers. 400×.

FIG. 48. Birefringent starch grain with weakly marked layers and missing amylogenic centre (station 10). 400×.

FIG. 49, 50. Tropical starch grains with irregular arrangement of layers, unlike those of arctic grains (station 17). 200×.

FIG. 51–53. Starch possibly derived from Florideae (see Fritsch, 1948) (station 13). 400×.

Figures 54–62

FIG. 54. Starch in macerated cell of an unidentified higher plant, stained blue after treatment with iodine (station 15). 250×.

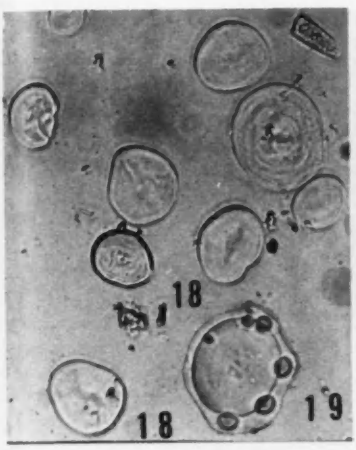
FIG. 55. Single cell of higher plant, containing starch (station 15). 250×.

FIG. 56. Extracellular starch, from 2000-m depth, ice island T-3, Arctic Ocean (station 5). 400×.

FIG. 57–61. Lens-like grains, presumably produced by Warnoviaceae, showing shallow depression (arrows) (Arctic Ocean, station 5). 1000×. *Fig. 57.* Grain in early stage of development, containing approximately 28–30 layers. *Fig. 58–61.* Grains with increasing numbers of layers up to 80. *Fig. 59.* Aberrant form of lens-like grain.

FIG. 62. Small and larger free starch grains (circled) among particles of silt, slightly bluish after addition of iodine; empty valva of benthic diatom *Campylodiscus* sp. on the right (station 8). 400×.

(*Figures 18–61 follow*)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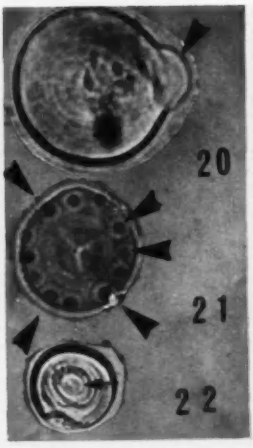
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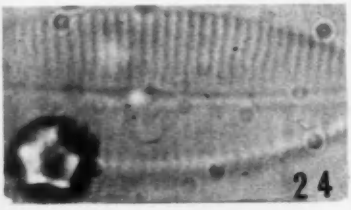
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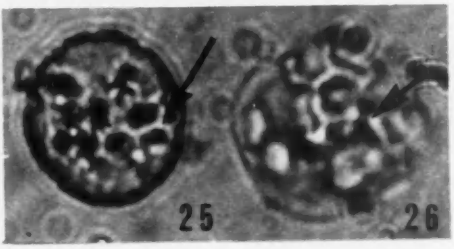
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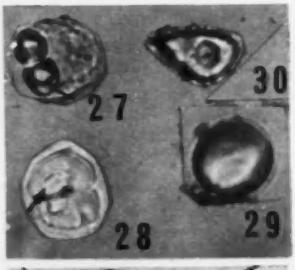
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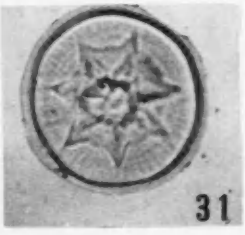
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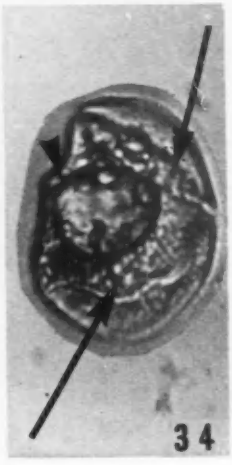
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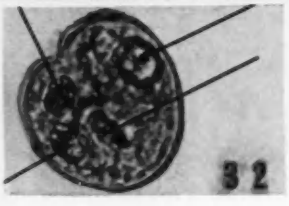
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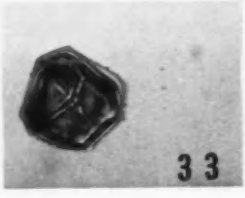
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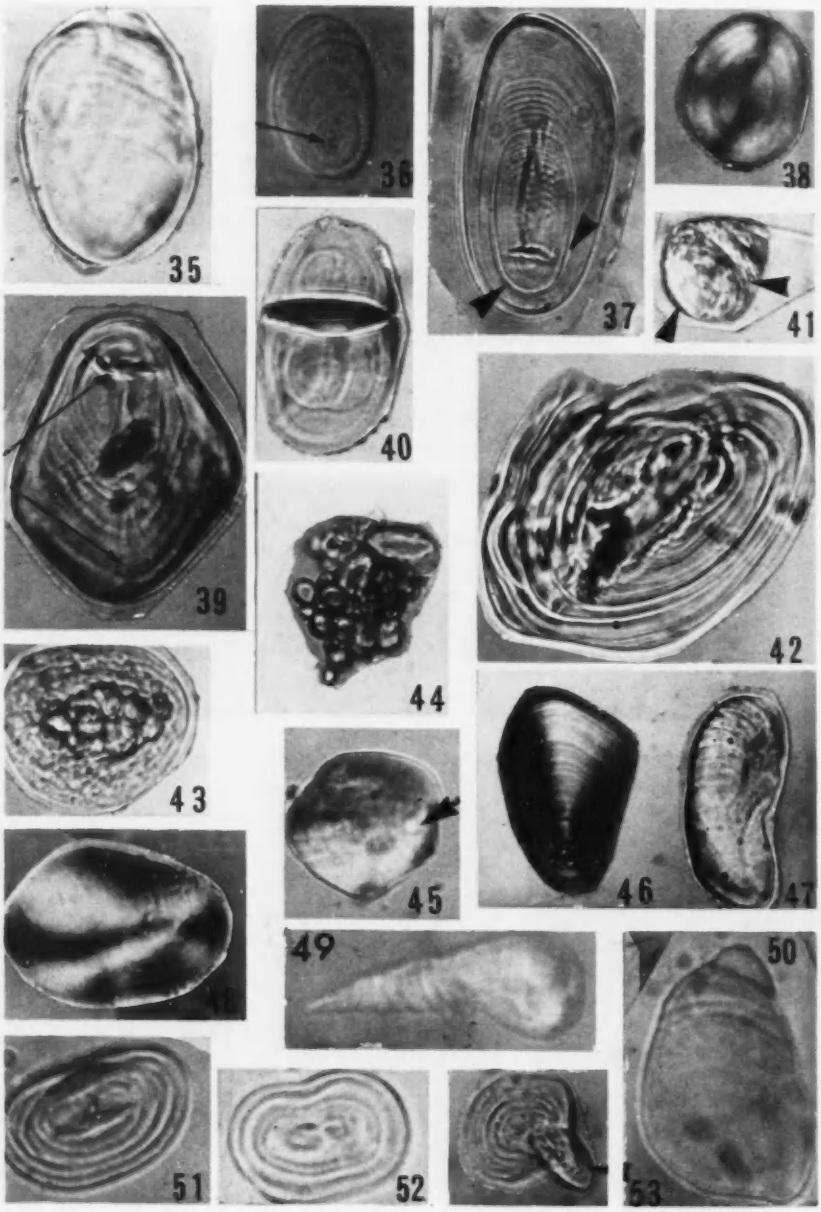
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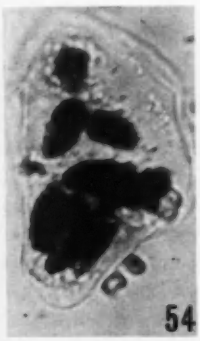
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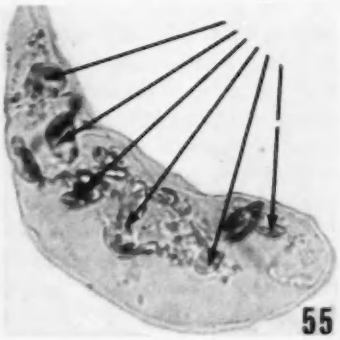
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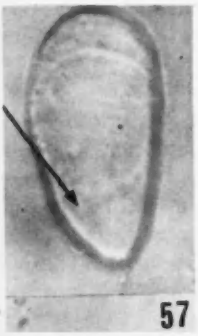
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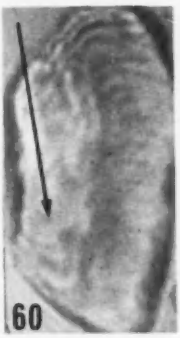
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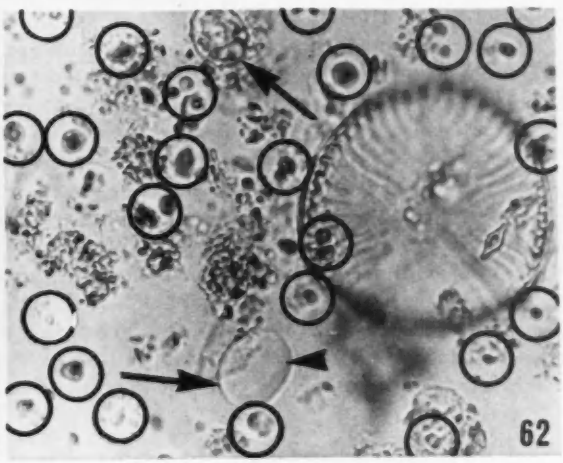
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diatoms appeared to be slightly digested the starch remained intact and was excreted from the cells.

Cylindrical faecal pellets (Fig. 23), often exceeding 500 μ long and containing hundreds of solid starch grains, are apparently voided by some unidentified planktonic herbivores probably grazing selectively on Gymnodinioideae. Since the starch in these faecal pellets was not digested, the herbivore was not an amylophage sensu strictu, but a "gymnodiniophage." Though no potential amylophage was found during my observations, there seems little doubt that some herbivorous animals exist in the plankton and amongst the mud-eaters of arctic regions in which starch of many kinds could be enzymatically digested. There is certainly plenty of starch deposited in arctic silts (Fig. 62) to supply such animals with food. It is significant perhaps that oysters fed with starch from higher plants increase in weight (Haven, 1965). Since lamellibranchs possess an amylase which can digest starch (Van Veel, 1961), arctic lamellibranchs, which occur in habitats where free starch is in abundance, may well utilize it.

Fate of Extracellular Starch

Hydrolysis of starch is influenced by the presence of electrolytes (Badenhuinzen, 1959), but the chemical changes are complex according to recent discoveries in carbohydrate chemistry reviewed by Whistler (1964). Since one third of marine bacteria were found by ZoBell (1946) to utilize starch, and since many other marine bacteria and fungi were also found to utilize starch (Kriss, 1963), destruction of free starch in the sea will always occur. Lytic processes in the starch of higher plants in heterogenic standing crops of arctic habitats may follow parallel lines, but some starch grains with dense layers (Fig. 24, 27–30, 32, 33) retain their shapes in sea water and are not readily destroyed. Examples are the ocellar lens-like grains, frequently found intact (Fig. 36, 37, 39, 48), which appear to be particularly resistant to degradation.

In amylolysis the grains swell (Fig. 18–22) and the layers are destroyed together with the amylogenic centre and the radial canals. These so-called canals (Badenhuinzen, 1959) should be re-named radial striae (Fig. 31, 39) since, in all grains I studied, they resulted only from differences in density and refraction of the starch matrix. Hydrolysis of starch extruded by some unidentified organisms revealed characteristic structures which otherwise would remain hidden (Fig. 25, 26, 31). Though in some starch grains lysis begins from a number of peripheral dark points (Fig. 21), in others it starts from a single point at the edge (Fig. 19, 20) or on the surface of the grain. In spite of hydrolysis, some starch grains (Fig. 51–53) can be recognized as originating from the group of algae Florideae (Fritsch, 1948). However, the producers of most of the starch grains in standing crops cannot at present be identified.

Starch Sedimentation

Plankton sedimentation occurs not only in sheltered waters (Grøntved and Steeman Nielsen, 1957). Repeated influxes of brackish waters into the

sea destroy Dinophyta and Chlorophyta, resulting in annual sediments (Willen, 1962). Similar conditions appear to control dispersal and starch sedimentation in arctic estuaries and along the coastal, and possibly offshore, waters. In the laboratory, extracellular starch grains of all sizes and states of fragmentation were found to settle on the bottom of sedimentation chambers, almost simultaneously with the phytoplankton. The smallest starch grains and phytoplankton cells settled more slowly. In nature, the differential sedimentation rate of starch grains results in a differential dispersal by water currents, similar to that shown by other organic detritus (Parsons, 1963). Though the vertical and horizontal distributions of standing starch crops were analyzed within the Arctic–Atlantic region, no regularity of occurrence was found owing to this constant mixing and dispersal in the plankton, absorption on the undersurface of ice, and settling in silts. In some surface sediments in which lysis appears to be inhibited, both in salt- and in freshwater environments, the fossilization of free starch would be expected.

Conclusions

Since planktonic autotrophs that produce starch occur in all seas, the distribution of such products must be related to the quantitative and qualitative occurrences of the organisms. Necrocenoses of Gymnodinioideae blooms are accompanied by liberation of extracellular starch in the marine environment. Climatic changes in geological times probably induced necrocenoses of the dinoflagellates recorded as paleomicrofossils (Downie et al., 1963). Though in such deposits starch has not been recorded, its presence should be expected, according to available data.

Sunny weather has been shown to be a primary factor in the depletion of marine nutrients by phytoplankton (S. Apollonio, personal communication), and in the subsequent destruction of the organisms by bacteria. Huge, heterogeneous standing crops of starch observed in some arctic habitats were formed by the many species of Gymnodinioideae present. The abundance of starch in suspension in the arctic marine environment in general appears to result from the deposition of many crops during not one but probably several consecutive seasons. Retarded amylolysis and cessation of bacterial and fungal metabolism in temperatures below 0 C over long periods probably permit survival of starch grains for a longer time than is possible in warm seas.

Since most marine phyto-detritus is derived from phytoplankton, its re-utilization by animals will tend to increase the efficiency of the conversion of plant carbon to animal carbon (Parsons, 1963). This probably applies mainly to starch in suspension. The increase of dissolved carbohydrates and other organic matter parallels the increase of diatoms (Marshall and Orr, 1964), which are the main producers of arctic biomass (Gran, 1930; Braarud, 1935; Brunel, 1962). Because of this, starch floating in arctic seas must be contributed mainly by Dinophyta, Chlorophyta, benthic algae, and some

higher plants. In the absence of quantitative data on starch in suspension and its derivatives dissolved in sea water as monosaccharides (Strickland, 1960), it may be presumed that the total weight of starch found in plankton, on the undersurface of sea ice, and in sediments must amount to incalculable millions of tons, and hence a significant factor in the bioeconomy of marine life.

The diversity of structure in starch of unicellular phototrophs suggests a long evolutionary history, such groups as the Warnoviaceae having evolved at the dawn of life in Paleozoic seas. Members of this family, which bear cnidocysts as in Coelenterata, generate reserve starch in the chromatophores and a unique lens-like starch grain in the melanosome pigment of the ocellus. The lens-like grain is structurally unlike any other starch form in the entire plant kingdom.

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NOTES

Influence of Light on Activity of Brown Trout (*Salmo trutta*)

Many workers have examined the activity patterns of fish and concluded that there is a regular variation in the level of activity during 24 hr. However, although the existence of these daily rhythms is well established, there is insufficient evidence to entirely explain how these patterns are controlled. A number of workers have correlated activity with the feeding cycle (Kuchin, 1900; Hoar, 1942; Oliphan, 1951; Hirata and Kobayashi, 1956; Hirata, 1957), but more recently Swift (1964) artificially fed brown trout and found no change in the activity pattern. He concluded from this work that the food requirements of a fish have little effect upon its activity, and suggested that environmental factors such as light were possibly the chief control factors. The present study was designed to examine further this suggestion that the effect of light could control the activity patterns of brown trout (*Salmo trutta*).

Experimental procedures.— Few "wild" fish become habituated to the presence of humans, and therefore it was considered that the technique of direct observation employed in many behavioural studies (e.g. Harker, 1953) was unsuitable, the presence of an observer probably disturbing the trout. Therefore it appeared preferable to record activity with an automatic monitoring device, of which a number already existed.

A copy was made of the Spoore's (1941) fish paddle, but pilot experiments soon showed that this device is rather insensitive. Also this apparatus, and other fish activity recorders which operate on the same principle (e.g. Davis 1963), can be criticised on the grounds that the paddle presents an obstacle to the movements of the trout. A more sophisticated device, Moore's (1961) 18-point fish recorder, was also considered and rejected, on the grounds that the high cost of the instrument made it a prohibitive proposition. Thus it was decided to adapt another existing technique, the light beam or "magic-eye" device, which has been used with success by ethologists studying the movements of terrestrial animals.

Because one can only use a light beam that does not disturb the animal being studied, one is limited to employing that part of the spectrum to which the fish are insensitive. Wald (1940) found that fish are insensitive to light above 600 mμ and in these experiments a limited spectrum was obtained by using Wratten (No. 70) filters which only pass light above 650 mμ. The use of such filters results in the employment of a restricted energy source, and the level of energy will be further reduced when passing the beam through water. Therefore both a high energy light source and a very sensitive photodetector are required if the apparatus is to function effectively.

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The high energy light source was obtained by using a commercial mounting which consisted of a 12-v d-c bulb arranged behind a lens in a focusable tube, and with this it was possible to produce a very narrow beam of light which was not heavily scattered when passing through water.

The photodetector circuit was required to register the interruption of the light beam by operating a relay. Normally this type of circuit incorporates a phototransistor as the light detector (e.g. Carter and Donker, 1963), but it was found that these were difficult to focus in the light beam and also were insensitive to low levels of light. After some consideration, it appeared that the cadmium sulphide cells were the most efficient type of photodetector that could be used with the circuit because they were easy to focus in the beam, and also capable of delineating between low light and complete darkness. The cadmium sulphide cell and its associated circuitry is shown in Fig. 1.

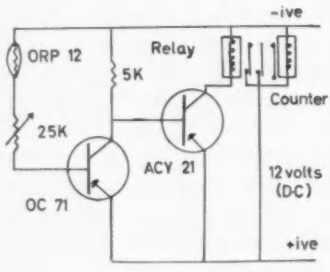

FIG. 1. The fish activity recorder. (Interruption of the beam causes the resistance of the cadmium sulphide cell to increase thus placing a greater load on the OC 71. The load increase causes amplification by the two transistors to a level sufficient to operate the relay.)

Because the fish cross the light beam at high speed, a very sensitive circuit was required, and this was developed after consultation with Mr J. Roach (Department of Chemistry, University of Exeter). The number of passes across the beam in a given time was recorded by connecting the relay to an electromagnetic counter or an Autovisor event recorder.

Three of the light beam and detector arrangements were mounted across an aquarium (1.22 × 0.61 × 0.61 m) which had been specially constructed. Its size was decided (*a*) by the width of the bench on which it was to be mounted; (*b*) by the size of the available slate for the base of the tank; and (*c*) by the fact that the detector could only receive sufficient radiant energy if placed within 1 m of the light source. The aquarium was mounted on the top of a 1-m concrete and slate bench to absorb any vibrations from movements near the experiment, and the sides of the tank were painted black in order that the movements of people were not visible to the fish. It was considered that such precautions prevented any disturbance effects, and this view was substantiated by the fact that there was no difference between experiments carried out during the week and those at weekends.

The setting of the light beams, 6, 32, and 51 mm from the water's surface midway along the length of the tank, was chosen after some preliminary observations had been made on the movements of the trout. It was found that

when they were inactive they remained very close to the bottom in a corner of the tank, but that during periods of activity the trout swam lengthways up and down the tank and it was usual for the fish to rise off the bottom and swim higher up in the water column.

Three fish were used in each experiment; this trebled the baseline of activity and thus amplified small changes in activity to an observable level. The activity of three batches of fish was examined for 24 hr under normal light conditions, constant darkness, continuous illumination, and reversed light conditions. Continuous illumination was obtained by mounting a 100-w, 240-v a-c, unreflected bulb about 1 m above the water's surface. Constant darkness was achieved by completely covering the top of the tank with polythene sheeting. By employing both of these techniques it was also possible to expose the trout to reversed light conditions.

All the fish to be used in these experiments were obtained by electric stunning from a Dartmoor stream in southwest England. In order to eliminate the effect of variations in maturity affecting the results, all the fish used were in the 2+ age-class (class data from Horton, MS, 1958). During the journey from the stream the fish were aerated with pure oxygen, and upon arrival at the laboratory they were immediately placed in the aquarium where they were left undisturbed for 48 hr to enable them to become accustomed to their new environment. The trout were then given a 24-hr period under the light condition to be used in the experiment before any observations were made upon the activity. It was considered that this period allowed the fish to recover from any disturbance resulting from the change in illumination.

Results.— Plots are given of the activity of one batch of fish under the various light conditions (see Fig. 2 and 3). These results are similar to those of the other two batches and the variation between the results was tested statistically by applying an F test (Simpson et al., 1960) to the number of passes across the light beams per hour by the three batches of fish under each light condition.

Under normal light conditions $P = <0.1\%$ and therefore it can be stated that there is a definite variation in activity during 24 hr. However, in the experiments under constant darkness and continuous illumination, $P = >5\%$ and therefore it would appear that activity is random under constant conditions. Again under reversed light conditions there is a definite variation in activity ($P = <0.1\%$) during 24 hr.

Conclusions.— Under normal conditions there is a definite activity pattern, the trout in all the experiments being most active between the period dusk until dawn; whereas under constant conditions the activity becomes more random, although the overall level of activity is higher under constant darkness than continuous illumination. Reversing the light conditions results in a change in activity (compared with that under normal conditions) so that the fish are again active during darkness and relatively inactive during illumination.

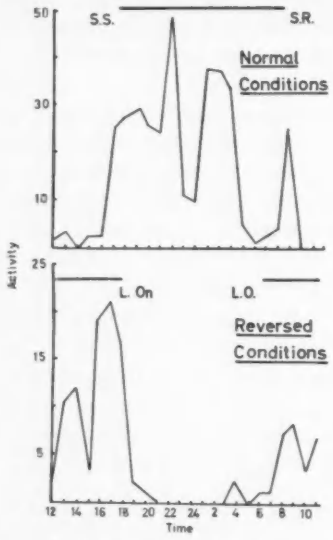

FIG. 2. The activity of the trout under normal and reversed light conditions. S.S. = sunset; S.R. = sunrise; L.On = light on; and L.O. = light off.

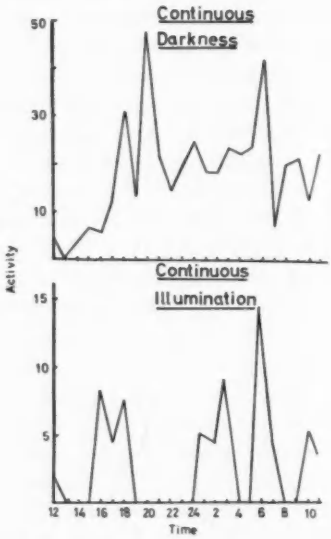

FIG. 3. The activity of the trout under constant conditions.

It is concluded from (*a*) the low level of activity under continuous illumination; (*b*) the higher level of activity during periods of darkness; (*c*) the increase in activity at sunset; and (*d*) the immediate decrease in activity upon illumination in the reversed light condition experiments, that the activity of brown trout is depressed during periods of exposure to daylight or artificial illumination.

Thus it is considered that these results substantiate the suggestion of Swift (1964) that light could be an important factor in the control of the activity pattern of *S. trutta*.

Discussion.— Although it is apparent that light plays an important role in the control of the activity pattern, it is less obvious how this control is applied. Davis and Bardach (1965) concluded from studies on the time coordinated prefeeding activity of killifish and bluegill that the LD′ cycle may act as a timegiver, thus assisting the establishment of a response to regular feeding by stabilising the phase of the biological clock. However, evidence to substantiate this theory is not forthcoming from this present study. In fact the rapid alteration of the activity pattern after only 24 hr under reversed light conditions would suggest that the effect of light is entirely exogenous and not at all related to the phasing of a biological clock.

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Estimation of the Age of Exploitation at a Given Fishing Mortality

According to the concept of eumetric fishing (Beverton and Holt, 1957) there is a particular age of exploitation or selection of gear which for a fish population will maximise the yield at a given fishing mortality rate (F). Apart from reading approximately the best age of exploitation for a given F from the eumetric fishing curve or from the equilibrium yield curve, no accounts are known to me giving a more precise method of estimating this important parameter. This note therefore gives a general algebraic solution of estimating the required age of exploitation.

The equation for yield in weight (Yw) developed by Beverton and Holt for a population in a steady state is

$$Yw = FRW_\infty e^{-M(t_p - t_\rho)} \sum_{n=0}^{3} \frac{\Omega_n e^{-nk(t_{p'} - t_0)}}{z+nk} \left[1 - e^{-(Z+nk)(t_\lambda - t_{p'})}\right] \quad \ldots\ldots(1)$$

where F = instantaneous fishing mortality rate, M = instantaneous natural mortality rate, Z = instantaneous total moratality rate (i.e. F + M), R = number of recruits entering the fishing ground, W_∞ = asymptotic weight, K = rate of deceleration in growth increments, t_0 = arbitrary age at zero length, t_p = age at which the fish enter the fishing ground, $t_{p'}$ = age at which the fish is first fully exploited, and t_λ = maximum exploitable age. In the above equation the growth in weight of the individual fish is isometric, i.e. the value of the exponent, γ, in the equation describing the length–weight relationship is 3.

The age of exploitation maximising the yield at a given F can be calculated by differentiating the equation 1 with respect to $t_{p'}$, equating to zero, and finally solving it for $t_{p'}$. But the resulting equation can be applied only when the growth is isometric. However, when the growth exponent is not 3, yield can be calculated accurately using the method of Jones (1957) or of Paulik and Gales (1964), but these models cannot be differentiated easily with respect to $t_{p'}$. Krishman Kutty (1968) gives a modified version of the Beverton and Holt equation from which the yield can be calculated fairly accurately when growth is not isometric. This equation states

$$Yw = FRW_\infty e^{-M(t_p - t_\rho)} \sum_{n=0}^{d-1} (-1)^n \frac{\Omega_n e^{-nk(t_{p'} - t_0)}}{Z+nk} \left[1 - e^{-(Z+nk)(t_\lambda - t_{p'})}\right] \quad \ldots\ldots(2)$$

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where Ω_n takes successively the values of 1, $\frac{\gamma}{1!}$ $\frac{\gamma(\gamma-1)}{2!}$,

$$\ldots \frac{\gamma(\gamma-1)(\gamma-2)\ldots(\gamma-(d-2))}{(d-1)!}$$

and d = number of binomial terms obtained by expanding the equation for weight at time t,

$$W_t = W_\infty [1-e^{-k(t-t_0)}]^\gamma \quad \ldots\ldots(3).$$

Equation 2 is differentiable and hence by differentiating 2 instead of 1 a general solution for calculating the $t_{p'}$ can be obtained for all values of γ. When γ is a positive integer the upper value $(d-1)$ in the summation term, $\sum_{n=0}^{d-1}$ in equation 2 will be equal to the value of γ. But when γ is not a positive integer, equation 3 will not terminate on expansion and d in equation 2 will assume a value equal to the number of binomial terms which would be sufficient for obtaining a fairly accurate estimate of yield.

Differentiating equation 2 with respect to $t_{p'}$,

$$\frac{dYw}{dt_{p'}} = FRW_\infty e^{-M(t_p-t_p)}\left[\frac{1}{Z} \cdot -e^{-Z(t_\lambda-t_{p'})} \cdot Z - \frac{\gamma_{(1)} e^{-k(t_p-t_0)}}{Z+k} \times \right.$$

$$-e^{-(Z+k)(t_\lambda-t_{p'})} \cdot (Z+k) + \frac{\gamma_{(1)} ke^{-k(t_p-t_0)}}{Z+k}\left\{1-e^{-(Z+k)(t_\lambda-t_{p'})}\right\}$$

$$+ \frac{\gamma_{(2)} e^{-2k(t_p-t_0)}}{Z+2k} \cdot -e^{-(Z+2k)(t_\lambda-t_{p'})} \cdot (Z+2k)$$

$$\left. - \frac{\gamma_{(2)} 2ke^{-2k(t_p-t_0)}}{Z+2k}\left\{1-e^{-(Z+2k)(t_\lambda-t_{p'})}\right\} \ldots\ldots\right] +$$

$$\left[\frac{1}{Z}\left\{1-e^{-Z(t_\lambda-t_{p'})}\right\} - \frac{\gamma_{(1)} e^{-k(t_p-t_0)}}{Z+k}\left\{1-e^{-(Z+k)(t_\lambda-t_{p'})}\right\}\right.$$

$$\left. + \frac{\gamma_{(2)} e^{-2k(t_p-t_0)}}{Z+2k}\left\{1-e^{-(Z+2k)(t_\lambda-t_{p'})}\right\} \ldots\ldots\right] \times$$

$$FRW_\infty e^{-M(t_p-t_p)} \cdot -M \quad \ldots\ldots(4)$$

where $\gamma_{(1)}$, $\gamma_{(2)}$, etc. are respectively $\frac{\gamma}{1!}$ $\frac{\gamma(\gamma-1)}{2!}$, etc. Putting $\frac{dYw}{dt_{p'}} = 0$, cancelling the common factor, $FRW_\infty e^{-M(t_p-t_p)}$ and after rearranging and simplifying, equation 4 is reduced to

$$e^{Z(t_\lambda - t_{p'})}\left[\frac{M}{Z} - \frac{\gamma_{(1)}\,(M+k)}{Z+k}e^{-k(t_{p'}-t_0)} + \frac{\gamma_{(2)}\,(M+2k)}{Z+2k}e^{-2k(t_{p'}-t_0)} \ldots\ldots\right]$$

$$= -\frac{Z-M}{Z} + \frac{\gamma_{(1)}\,(Z-M)}{Z+k}e^{-k(t_\lambda - t_0)} - \frac{\gamma_{(2)}\,(Z-M)}{Z+2k}e^{-2k(t_\lambda - t_0)} + \ldots\ldots(5).$$

The factor (Z–M) or F being common in all terms of RHS and the sign of all the terms alternates, equation 5 can be written as

$$e^{Z(t_\lambda - t_{p'})}\sum_{n=0}^{d-1}(-1)^n\frac{\gamma_{(n)}\,(M+nk)}{Z+nk}e^{-nk(t_{p'}-t_0)} = -F\sum_{n=0}^{d-1}(-1)^n\frac{\gamma_{(n)}e^{-nk(t_\lambda - t_0)}}{Z+nk} \ldots\ldots(6).$$

The exact solution of equation 6 is not easy since $t_{p'}$ appears in exponential terms of different powers. However, it can be calculated fairly accurately by successive approximation first by assigning two values to $t_{p'}$ and getting two estimates of LHS of equation 6 so that one is more and the other is less than the RHS value. Then by linear interpolation the first approximate value of $t_{p'}$, say $t_{p'1}$ is calculated. An estimate of LHS is made again after inserting the $t_{p'1}$ in equation 6 and if it is not identical with RHS, $t_{p'2}$ is calculated as before by interpolation. This procedure is continued until the equation is satisfied. The $t_{p'n}$ equalising both sides of equation 6 is the required $t_{p'}$ that will maximise the yield at a given F. Generally a fair estimate of this $t_{p'}$ can be obtained by three or four interpolations.

Using the above method the optimum $t_{p'}$ has been calculated for F = 0.73 for the North Sea plaice. The required data taken from Beverton (MS, 1954) are M = 0.1, Z = 0.83, t_p = 3.72 years, t_λ = 15 years, K = 0.095, t_0 = –0.815 years, W_∞ = 2867 g and γ = 3. The optimum $t_{p'}$ for F = 0.73 is found to be 10.4219 years although from the equilibrium yield curve Beverton concludes that the optimum $t_{p'}$ is 10 years. From Table I it can be seen that

TABLE I. Yield per recruit $\frac{(Yw)}{R}$ for plaice when F is 0.73 for values of $t_{p'}$ around the estimated best age of exploitation of 10.4219 years.

F = 0.73	
$t_{p'}$ years	$\frac{Yw}{R}$ g
10.0	413.10
10.40	413.38
10.42	414.42
10.4219	*414.42*
10.43	414.44
10.44	414.40
10.50	414.39

10.4219 is a more accurate estimate of the optimum $t_{y'}$ which will maximise the yield at F = 0.73.

Equation 6 can be used to solve for $t_{y'}$ even when the growth exponent γ in length–weight relation is not three. When γ is three, the upper value in the summation term $\sum_{n=0}^{d-1}$ will be three as also in equation 2 and the values of the binomial coefficients γ_n will be successively 1, 3, 3, and 1. When r is not a positive integer, the accuracy of the optimum $t_{y'}$ for a given F can be increased by increasing the number of terms in equation 6 although it is seldom necessary to take more than 8 or 10 terms. However, if a digital computer is available the total number of terms in equation 6 can be considerably increased.

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Preliminary Results in the Fertilization of Eggs with Frozen Sperm of Atlantic Salmon (*Salmo salar*)

A recent report from this laboratory described the use of protective agents to achieve sub-zero, unfrozen, storage of sperm of Atlantic salmon. Several attempts to freeze sperm of this species since 1963 have been unsuccessful, but occasionally a very small percentage (ca. 0.5–2) of eggs have been fertilized. There was no evidence that these resulted from contamination before, during, or after fertilization. In 1967 it was decided to be particularly careful to avoid contamination and carefully record any further incidence of fertilization with frozen sperm. The results are the subject of this note.

During this season's investigations, ethylene glycol (EG) was used as a protective agent, being dissolved in the previously described (Truscott et al., 1968) "modified Cortland medium" (m.C.), with or without a further added solute. This final solution, or extender, was added to an equal volume of sperm over a period of 1 hr to give the required final concentration.

The search for useful cryoprotective agents has covered a wide range of compounds (Doebbler, 1966). Apart from such obvious properties as high solubility in water, cell permeability, and nontoxicity, one feature stands out, namely, the strong hydrogen-bonding capability of many solutes affording good cryoprotection. Two such groups of compounds which have been found useful in certain instances are sugars (Doebbler and Rinfret, 1962) and amino acids (Doebbler and Rinfret, 1962; Martin, 1965). Of these, lactose and serine were chosen for investigation, the former in view of its reported protective action in freezing bull sperm (Nagase, 1966), serine because of an expected high hydrogen-bonding capacity. An amino acid mixture (casein hydrolysate) was also used.

The basic extender used was 5% EG in m.C., with variations in the concentration of further added solute. The cooling rates were varied as follows:

(A) "Instantaneous," i.e. the samples were contained in a thin, liquid-proof aluminum packet and plunged into liquid nitrogen.

(B) One minute per degree fall in temperature from 2 to −25 C.

(C) Eight minutes per degree fall from 2 to −10 C, followed by 4 min per degree from −10 to −25 C.

(D) Eight minutes per degree from 2 to −10 C with a 12-hr equilibration period at −6 C, i.e. just above the freezing point, followed by four degrees per minute from −10 to −25 C. All samples froze at −10 C or higher and were held for half an hour at the final temperature. Subsequent thawing was effected at 3 C.

Motility was examined by dilution in 20% sea water and observation under phase contrast at 630× magnification. Fertility was determined by adding the sample to approximately 200 eggs, washing twice with cold fresh water, and incubation for about 12 days in running, aerated fresh water at

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TABLE I. Per cent of eggs fertilized with frozen salmon sperm and corresponding sperm motility under a variety of freezing conditions.

	Cooling rates[b]							
	A		B		C		D	
Addition[a]	M[c]	F[c]	M	F	M	F	M	F
None	0	0	0	0				
2.5% EG	0	0	0	0				
2.5% serine	0	0	0	2	1	4	1	4
2.5% lactose	0	0	1	0	1	12	1	0
2.5% amino acid mixture[d]	0	0	1	0	0	0	0	0

[a]Sperm was mixed with an equal volume of extender which contained the indicated addition. Extender was 5% ethylene glycol (EG) in "modified Cortland medium" (see Truscott et al., 1968).
[b]See text for description of cooling rates A–D.
[c]M — motility: 0 — zero motility, 1 — very weak motility; F — percentage fertility.
[d]Amino acid mixture used was casein hydrolysate.

7 C; fertilized eggs developed a characteristic white streak (notochord) on addition of 8% acetic acid.

Neither 5 nor 7.5% EG in m.C. alone was successful in allowing survival of frozen sperm. Some small success was achieved, however, by further addition of solute to the extender and use of a slow cooling rate; sperm stored in the presence of 2.5% serine in 5% EG in m.C. gave fertilities of 2% at rate B and 4% at rates C and D, whereas 2.5% lactose in 5% EG in m.C. gave 12% fertility at rate C. A slight amount of motility was seen in each of these cases except for the sample with 2.5% serine at rate B, in which no motility was seen. The same weak motility was also observed in samples with 2.5% lactose in 5% EG in m.C. frozen at rate D and 2.5% amino acid mixture in 5% EG in m.C. at rate B, but there was no corresponding fertility. The very fast rate (A) gave neither motility nor fertility in any example, but produced marked clumping of the spermatozoa. Reduction of the concentration of serine, lactose, or amino acid mixture to 1% or its increase to 3% did not allow successful recovery of active sperm after freezing by any of the four cooling rates.

Successful freezing and thawing of cod sperm in this laboratory (M. S. Mounib et al., in preparation) will, it is hoped, yield fundamental information helpful in clarifying the problems associated with freezing of salmonid sperm.

NOTE ADDED IN PRESS: A recent press release (March 21, 1968) from the United States Department of the Interior stated that scientists at Oregon State University fertilized eggs with cryopreserved sperm. Fertility was reported as only 0–18%. We shall attempt to confirm our results later this

year but release them at this time in the hope of stimulating research on this challenging problem.

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An Estimate of Fecundity of the Winter Flounder, *Pseudopleuronectes americanus*[1,2]

In spite of the winter flounder's importance to the marine economy of New England and the Maritime Provinces, fecundity of the species is known only through isolated estimates. Bigelow and Schroeder (1953) reported upper and lower values of 0.5 and 1.5 million eggs per female, presumably based on the data of Smith (1898) in Massachusetts. Fecundity of Rhode Island stocks has been estimated by Saila (1961); of Newfoundland stocks by Kennedy (MS, 1964).

The following analysis is based on a sample of 30 mature females collected by the Massachusetts Division of Marine Fisheries by otter trawl off Ellisville, Sandwich Creek, and White Cliffs, Massachusetts, on March 22, 1966. Judging from the distention of the ovaries and the appearance of the eggs, the fish in this collection were in various stages of late ripening. Spent fish were reported in collections made during the following month. No ripe age 2 fish were found. Their ovaries, weighing up to 2.3 g, contained immature eggs.

Ovaries were preserved in 10% formalin. Eggs were enumerated following the procedure of Lehman (1953), in which the numbers of ova found in six ovarian samples (in my case, eight) from each fish were averaged, and the mean used to estimate individual fecundity. Egg diameters were read in ocular units on a fish scale projector, then converted to millimeters. Ages were estimated by best agreement of annuli on scales, otoliths, and interopercular bones of each fish. Since the collection date of the sample roughly coincides with the birthday of winter flounder in the Massachusetts area (Pearcy, 1962, p. 40), age 3, 4 and 5 fish are those having just completed their 3rd, 4th, and 5th years of life, respectively. Figure 1 shows size distribution by age for the sample.

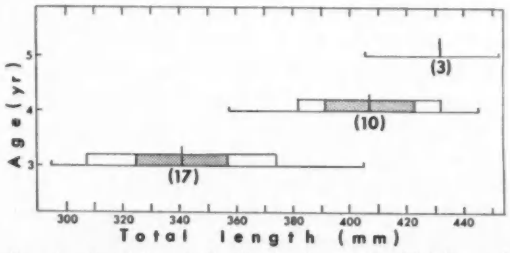

FIG. 1. Total length distribution by age for 30 fish used in fecundity estimates, showing range and mean, with one standard deviation and two standard errors on either side of mean. Numbers of specimens in parentheses.

[1]Based on a thesis submitted in partial fulfillment of requirements for the degree of Master of Science at the University of Massachusetts, Amherst, Mass.

[2]Contribution No. 5 of the Massachusetts Cooperative Fishery Unit, jointly sponsored by the U.S. Bureau of Sport Fisheries and Wildlife, the Massachusetts Division of Fisheries and Game, the Massachusetts Division of Marine Fisheries, and the University of Massachusetts.

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Enumeration of ova.— Three age 3 fish representative of the size-range for their age (290, 351, and 395 mm) were used in an analysis of variance to test the differences in ova density among fish, among locations within ovaries (Fig. 2), and between paired ovaries. Although ovaries of the blind side were consistently larger than those of the eyed side, there were no differences in ova density between paired ovaries, nor among the four locations within ovaries (Table I). However, considerable differences were observed from one fish to

TABLE I. Analysis of variance of ova density.

Source of variation	df	Mean square	F
Among fish	2	48,142,240	35.719**
Among ovarian locations	9	1,347,811	1.630
Between paired ovaries	12	826,993	1.675
Total	23		

**Signficant at $P = 0.01$

another, as is evident from the high F value. Differences were associated primarily with egg size.

Among the 30 fish in the sample, ova density ranged from 6082 eggs per gram of ovary to 18,963 eggs per gram, with an average of 10,595 eggs per gram. Total egg count ranged from 0.435 million in an age-3 fish to 3.329 million in age-5 fish. The regression equation for the relationship of fecundity to fish weight (Fig. 3)

$$\log F = 0.0697 + 1.0659 \log W$$

fits the data adequately. The equation of Saila (1961, p. 102), based on a sample of 46 fish from Narragansett Bay in January 1958, yields somewhat lower egg numbers. A 1000-g fish, for example, would contain 1.219 million eggs by Saila's estimates, and 1.851 million by mine.

Egg size.— A random sample of at least 30 eggs from each fish was measured. Individual diameters of eggs hardened in formalin ranged from 0.33 to 1.00 mm, with an overall mean of 0.61 mm. Distributions of egg sizes within samples were unimodal (e.g., Fig. 4). Egg size was uniform in some specimens, variable in others; coefficients of variation ranged from 6.9 to 14.1%. High variability, which often reflects reduced food supply during gametogenesis, may be adaptively important by allowing the embryos to hatch asynchronously (Nikolsky, 1962).

No significant correlation was found between mean egg size and fish size ($r = 0.312$, $n = 30$). Egg size differed among the three age-groups ($F = 7.314$, df = 2 and 27). A comparison among means by Tukey's test (Snedecor, 1956, p. 251) showed that mean egg size of age-3 fish was smaller than that of age 4 or 5 fish ($p < 0.05$). Asynchronous ripening within an age-group no doubt contributed to the heterogeneity of egg size among individuals of that group.

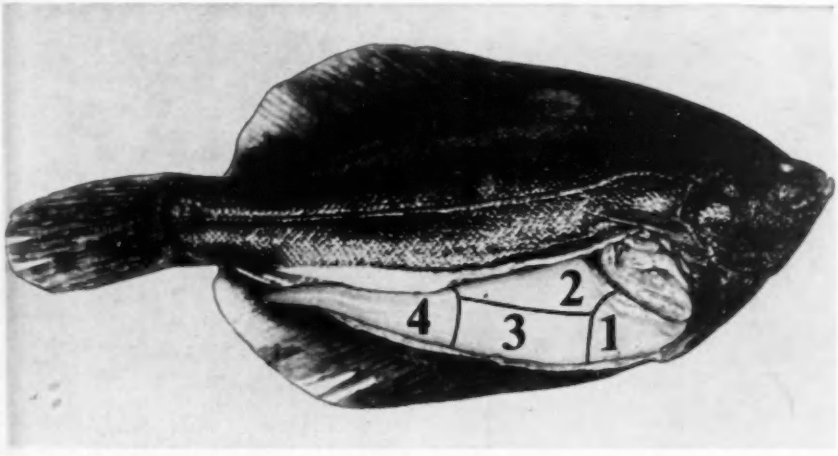

FIG. 2. Winter flounder ovary, eyed side, showing four regions tested for differences in ova density.

Topp — J. Fish. Res. Bd. Canada

FREQUENCY
Log10 Fecundity

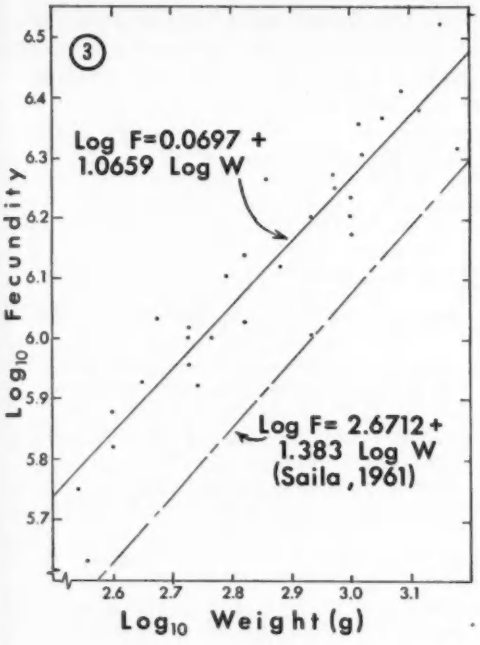

FIG. 3. Scatter diagram showing relationship of logarithm of fecundity to logarithm of fish weight. $n = 30$.

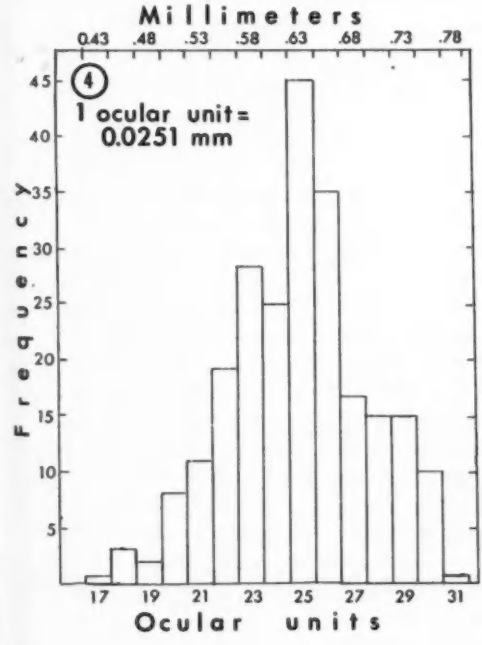

FIG. 4. Distribution of egg diameters for 240 eggs from an age-4 fish 451 mm TL.

I wish to thank Al Petersen of the Massachusetts Division of Marine Fisheries for obtaining the fish for this study. Laboratory facilities were provided by the Massachusetts Cooperative Fishery Unit.

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Asymptotic Growth: An Example of Nonsense Disguised as Mathematics

> *"In the space of one hundred and seventy-six years the Lower Mississippi has shortened itself two hundred and forty-two miles. That is an average of a trifle over one mile and a third per year. Therefore, any calm person, not blind or idiotic, can see that in the Old Oölitic Silurian Period, just a million years ago next November, the Lower Mississippi River was upward of one million three hundred thousand miles long, and stuck out over the Gulf of Mexico like a fishing rod. And by the same token any person can see that seven hundred and forty-two years from now the Lower Mississippi will be only a mile and three quarters long, and Cairo and New Orleans will have joined streets together, and be plodding comfortably along under a single mayor and a mutual board of aldermen. There is something fascinating about science. One gets such wholesale returns of conjecture out of such a trifling investment of fact."*
>
> *Mark Twain — Life on the Mississippi*

A person seeking some mathematical curve to approximate growth data often chooses the von Bertalanffy (1938) or negative exponential curve for the job. This is reasonable both because it is simple and often fits the data about as well as anything else, and because the nature of a lot of growth models is such that they will generate an exponential curve or some related curve such as the logistic.

The now familiar notation for the von Bertalanffy curve is

$$\text{length at age } t = L_\infty [1 - \exp (K (t - t_o))] \qquad \ldots\ldots (1)$$

and has three parameters, L_∞, K, and t_o. Passing K and t_o by, I propose to discuss L_∞, commonly interpreted as the maximum length of the fish or other organism. It is a common, perhaps even standard, practice to fit a von Bertalanffy curve to a set of growth data and report the L_∞ thus obtained as the maximum size. This procedure, however, can be dubious.

Consider this business of maximum length in terms of a couple of sets of growth data, heights of human males after Thompson (1948) shown in Fig. 1, and lengths of cod after Graham (1933) shown in Fig. 2.

In the first instance cessation of growth is known from common acquaintance with the beast, the idea of maximum size makes sense, and the maximum height is well defined by the data at a little over 170 cm (5 ft 7 in) for these Belgian males.

In the case of the cod, the growth curve can be reasonably well approximated by a straight line although there is some small visible curvature. The data show a nearly constant growth rate of about 14 cm per year and it is

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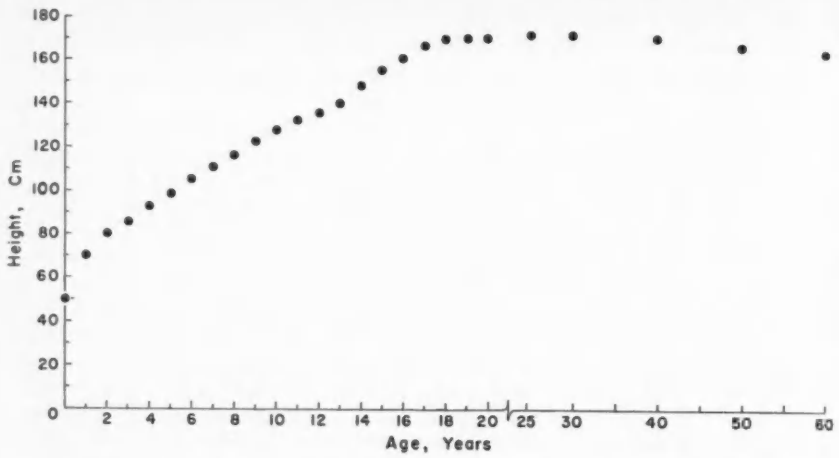

FIG. 1. Growth of Belgian human males. Source: Thompson (1948, p. 90)

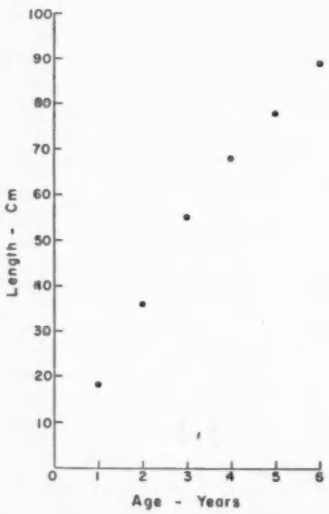

FIG. 2. Growth of cod. Source: Graham (1933).

not at all evident from the data alone at what length the growth stops, or even that it stops at all, and I believe that anyone who claims that this putative maximum is, say, the von Bertalanffy L_∞ (132 cm) (Beverton and Holt, 1957, p. 286) is either foolhardy or fortune telling.

In the case of the human data then, there is no objection to fitting an appropriate growth curve and reporting L_∞ or some equivalent symbol as the "average maximum height" or some such words. But I maintain that

however well the von Bertalanffy curve may fit the cod data, and in fact the fit is good, it is misleading to the reader to report any value for L_∞ at all, particularly if accompanied by an intimation that it represents the maximum size the fish can or does attain.

It is very easy to construct examples of the danger of extrapolation though these are apt to be artificial. Suppose that in the growth data given for human males only age classes 1 through 13 were available and the reckless endeavour by von Bertalanffy curves and mathematical magic to obtain L_∞, the size of the adult male (?). How to fit a von Bertalanffy curve is a knotty subject which I do not altogether understand, but I have picked two of the stronger contenders for a good method and fitted curves to the truncated growth data. (The actual methods were the fitting of a Walford (1946) line by least squares and an iterative least squares procedure (Allen, 1966a, b), but these details are not germane to the main line of thought.) The two methods yield L_∞ = 195 cm (6 ft 5 in) and L_∞ = 211 cm (6 ft 11 in) respectively. The divergence from the correct 170 cm might be excessive sampling error, but as both estimates are 2.5 or more standard errors therefrom, it is probably not sampling error which is the trouble in this case. In point of fact the real trouble is not mathematical and is well known, especially to parents of adolescents: the human growth pattern changes at about age 13.

In principle this sort of thing might happen whenever any growth curve, von Bertalanffy or other, is forced on inappropriate data. The forcing of curves certainly happens and may even be common. The first example I happen to find is some growth data for the petrale sole (*Eopsetta jordani*) presented by Ketchen and Forrester (1966, p. 111). Figure 3 is a plot of growth against size. This is equivalent to the standard Walford plot but exhibits curvature rather better. Although this plot shows consistent curvature, the authors used the von Bertalanffy curve in their analysis.

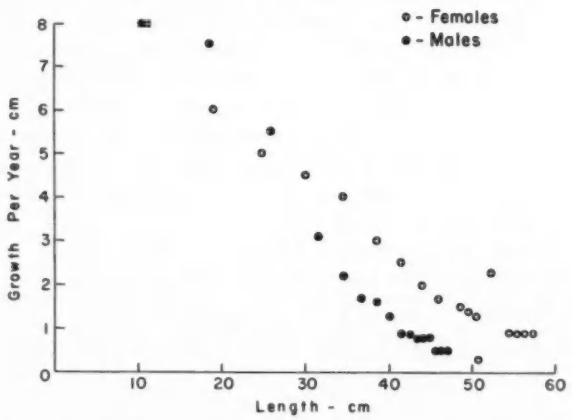

FIG. 3. Modified Walford plot of the petrale sole. Source: Ketchen and Forrester (1966).

However, Ketchen and Forrester do not fall into the trap of attempting a serious interpretation of the L_∞'s so calculated. Despite what I have said, I do not think that is an important danger in this study or any other. Indeed, to expect anything so blatant is to misunderstand the role of theory in science. More important is the distorted point of view engendered by regarding L_∞ as a fact of nature rather than as a mathematical artifact of the data analysis.

In comparative population studies one is tempted to tabulate L_∞ for a lot of species and environments and start comparing. This certainly calls for caution. If the L_∞'s are extrapolated from a lot of truncated growth data, the sampling error will be considerable at best, and the whole set of L_∞'s completely meaningless at worst. One example of such a comparative population study is Beverton and Holt's (1959). It includes, by the by, the 132 cm L_∞ for cod taken, apparently, from their own earlier analysis of Graham's data, tabled with no special comment, and not even rounded to 130 or 135 cm.

This same study contains an example of the way mathematical spectacles can distort an outlook in the remark: "The parameters T_{max} and M are, of course, closely linked on mathematical grounds; there is no reason why L_∞ and K should be, but it appears from the data that they are fairly closely correlated (inversely) although there are some important exceptions." (T_{max} and M are of no concern here.) However the von Bertalanffy K and L_∞ are mathematically linked; if K is zero, necessarily L_∞ is infinite. K among other things is a measure of curvature, and a large curvature relates to a small L_∞ in rather the same way that you have to bend a wire more sharply to make a small loop than a big one. If a growth curve is roughly straight as with Graham's cod and some other heavily fished species, some small apparent curvature, introduced by sampling error, will not merely be related to L_∞, it might cause it! Whether or not this is the actual cause of the observed correlation is irrelevant to this polemic. What does matter is the role a conceptual framework may have played in a possible cause being overlooked. Indeed, I believe the relation described above is obvious as soon as you consider K and L_∞ as descriptive summaries of data rather than as laws of nature.

For better or worse the use of mathematical tools in biology is growing. Any tool can, and occasionally will, be misused. A mathematical tool which the author believes is misused has been discussed. The unexpected point, to the author, is that the purported misuse seemingly did not come about because the user could not understand the mathematics since the mathematics is simple and the mistake not mathematical.

Thus the moral: That the main danger in a mathematical tool lies in the user's not understanding the mathematical foundations is obvious, but not necessarily true.

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